Am. J. Trop. Med. Hyg., 86(6), 2012, pp. 1018–1025
doi:10.4269/ajtmh.2012.11-0671

Facilitated Molecular Typing of Shigella Isolates Using ERIC-PCR

Margaret Kosek,* Pablo Peñataro Yori, Robert H. Gilman, Henry Vela, Maribel Paredes Olortegui, Cesar Banda Chavez, Maritza Calderon, Juan Perez Bao, Eric Hall, Ryan Maves, Rosa Burga, and Graciela Meza Sanchez

Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; Asociación Benéfica PRISMA, Lima, Peru; United States Navy Medical Research Unit, Lima, Peru; Regional Hospital, Loreto, Peru

Abstract. To evaluate the performance of enterobacterial repetitive intergenic sequence-based polymerase chain reaction (ERIC-PCR) typing versus the current standard for the typing of Shigella pulsed gel electrophoresis (PFGE), we typed 116 Shigella isolates from a village in an endemic setting over a 20-month period using both methods. PFGE identified 37 pulse types and had a discrimination index of 0.925 (95% confidence interval = 0.830–1.00), whereas ERIC-PCR identified 42 types and had a discrimination index of 0.961 (95% confidence interval = 0.886–1.00). PFGE and ERIC-PCR showed a 90.4% correlation in the designation of isolates as clonal or non-clonal in pairwise comparisons. Both systems were highly reproducible and provided highly similar and supplementary data compared with serotyping regarding the transmission dynamics of shigellosis in this community. ERIC-PCR is considerably more rapid and inexpensive than PFGE and may have a complementary role to PFGE for initial investigations of hypothesized outbreaks in resource-limited settings.

INTRODUCTION

Members of the genus Shigella are non-motile gram-negative bacilli that are classified into four species groups: *Shigella dysenteriae* (group A; 12 serotypes), *Shigella flexneri* (group B; 10 serotypes), *S. boydii* (group C; 18 serotypes), and *S. sonnei* (group D; 1 serotype). Shigella is currently a serious burden on the health of children in impoverished regions and a significant cause of severe diarrhea in travelers[1,2] and military personnel.[3,4] The organism has a very low infectious dose, a well-documented ability to cause sustained outbreaks from a wide variety of sources, and high rates of infection among close contacts. Outbreaks, either intentional (like a contamination event in the United States)[5] or non-intentional (such as the outbreak in Rwanda in 1994),[6] cause severe disease and are difficult to control. Rapid and accurate typing of patient isolates is necessary to understand transmission dynamics and control the spread of disease.

A wide variety of typing systems have been used to examine and compare isolates of Shigella species for outbreak investigation and taxonomic characterization. Previously used methods include biotyping; phage typing; plasmid analysis with or without endonuclease restriction; antibiotic sensitivity profiles; serotyping, ribotyping; restriction fragment length polymorphisms (RFLPs) of genomic target sequences of IpaH,[7] mglB, and gnd[8]; DNA hybridization strategies for plasmid and chromosomal elements[9]; multiplex polymerase chain reaction (PCR) of the O antigen gene cluster[10]; pulsed-field gel electrophoresis (PFGE)[9]; PCR amplification of enterobacterial repetitive intergenic sequences (ERIC-PCR)[11] or repetitive extragenic palindromic elements (REP-PCR)[12]; and randomly amplified polymorphic DNA (RAPD-PCR).[13] Additionally, colicin typing has been used for *S. sonnei*. In general, genomic- or chromosomal-based strategies are preferred over plasmid typing strategies, because plasmids may be lost. We chose to compare ERIC-PCR with the standard typing strategy for gram-negative enterics because of favorable assessment of discriminatory ability compared with PFGE in a small previous study typing 20 *S. sonnei* isolates.[11] ERIC-PCR is easier to perform, faster, and requires significantly less specialized equipment and reagents compared with PFGE. We compared the two tests on a diverse set of isolates representing all four major serogroups from an area where shigellosis is highly endemic. The success of ERIC-PCR as a simplified typing strategy for a wide and progressively expanding number of organisms makes this strategy a tenable one for hospital-based epidemiology or regional epidemiology, where a highly reproducible typing strategy that requires minimal disease-specific reagents is a desirable feature.

MATERIALS AND METHODS

Bacterial isolates. Shigella isolates for typing were obtained from children with diarrhea and their household contacts in a single rural village with a population of 3,543 individuals in the Peruvian Amazon, where shigellosis in highly endemic (this village is under active surveillance for diarrheal disease).[14] All isolates underwent standard biochemical testing and were typed into serogroups. Shigella B (*flexneri*) isolates were then serotyped. All serological typing was performed using Denka-Seiken antisera (Tokyo, Japan). The 116 isolates selected for testing were a convenience sample from a reference library of 218 strains collected in a 20-month period. Shigella isolates were characterized as being derived from uncomplicated diarrhea (non-dysenteric) if the individual had three or greater unformed or liquid stools in a 24-hour period, dysenteric diarrhea if the patient met the case definition for diarrhea and the laboratory technician noted the presence of gross blood in an unformed stool of a participant meeting the case definition of diarrhea, and asymptomatic if derived from a participant who did not meet the case definition for diarrhea at the time that the sample was given. All patients who participated in the study did so with informed consent, and the research protocol was reviewed and approved by human research review committees at the Johns Hopkins Bloomberg School of Public Health, Asociación Benéfica PRISMA, and the US Navy Medical Research Unit in Lima, Peru in compliance with all applicable federal regulations governing the protection of human subjects.

ERIC-PCR. Single colonies were streaked out on tryptic soy agar (TSA) and grown out overnight; 5–10 colonies were transferred,

*Address correspondence to Margaret Kosek, Department of International Health, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe St. w5515, Baltimore, MD 21205. E-mail: mkosek@jhsph.edu

and DNA was extracted using the standard tissue protocol of QIAamp DNA mini kit (Qiagen, Valencia, CA) with a lysis time of 5 hours. DNA was quantified using a λ-marker (Invitrogen, Carlsbald, CA) to titrate to 15 ng target DNA. Quantification of DNA enhanced reproducibility and the number of bands produced. DNA was added to a reaction mixture of 1 U recombinant Taq polymerase (Invitrogen, Carlsbald), 20 mM Tris·HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$ (Gibco), 250 μM each deoxynucleotide triphosphates, 0.5 μM primer ERIC 1 5′-GTGAATCCCCAGGAGCTTACAT-3′, and water for a total reaction volume of 50 μL. The amplification program used was the program by Liu and others[11] with denaturation at 95°C for 5 minutes; 4 cycles of low stringency amplification at 94°C for 1 minute, 26°C for 1 minute, and 72°C for 2 minutes; 40 cycles of higher stringency at 94°C for 30 seconds, 40°C for 30 seconds, and 72°C for 1 minute; and then a single final stage at 72°C for 10 minutes. All gels were run with a negative control with no DNA and water to achieve the standard reaction volume. Products were resolved by agarose gel electrophoresis in 2% agarose in Tris·acetate-(ethylenedinitrilo)tetraacetic acid (EDTA) buffer with ethidium bromide at 50 V for 5 hours and visualized by ultraviolet (UV) transillumination. Gels were photographed and digitized with a Gel Doc 2000 system. Images were imported into Phoretix Gel analysis software v 5.2 (Nonlinear Dynamics, Newcastle, United Kingdom), and retardation factor corrections using two markers per gel were used to improve intragel and intergel measures of band size. Lane images were then imported into the Phoretix database, and an unweighted pair group average (UPMGA) diagram was constructed using Dice coefficients (Figure 1A). Cluster groups with ≥ 85% similarity were identified as identical pattern types.

PFGE. The standardized laboratory profile for the molecular subtyping of *S. sonnei* of FoodNet was strictly followed. Briefly, a single colony was streaked on TSA and grown overnight. Bacteria were suspended in TE buffer (100 mM Tris, 1 mM EDTA, pH 8.0), and concentration was measured and standardized between 0.9 and 1.3 using a spectrophotometer at 610 nm. Proteinase K (0.5 mg/mL; Invitrogen) was added to 0.4 mL bacterial suspension and cast in 1% SeaKem Gold and 1% sodium dodecyl sulfate (SDS) agarose in 10 mM Tris and 1 mM EDTA (pH 8); it was then allowed to solidify in a disposable plug mold (Biorad Laboratories). Lysis was done in 50 mM Tris and 50 mM EDTA (pH 8.0) with 1% sarcosyl with 0.1 mg/mL Proteinase K for 5 hours at 54°C in a shaking water bath at 200 rpm. Plugs were washed two times in sterile Ultrapure water with agitation for 15 minutes and then washed four times with TE buffer warmed to 50°C. Plugs were incubated in TE buffer for 15 minutes at room temperature. TE buffer was removed and replaced with fresh buffer and 50 U XbaI per sample and incubated in a 37°C water bath for 5 hours. Plugs were loaded into wells in a 1% SeaKem Gold Agarose in 0.5× TBE (Tris-Borate-EDTA) buffer. Electrophoresis was done on a Bio-Rad CHEF-II apparatus for 22 hours with an electric field of 6 V/cm, and the pulse time was increased from 2.2 to 54.2 seconds. A λ-ladder (Biorad) was used as a molecular size marker, with two being run on the extremes of each gel to correct band measures for slight gel distortions. Gels were stained with ethidium bromide for 15 minutes in a covered container and then destained for 10 minutes. The image was digitalized using a Gel Doc 2000

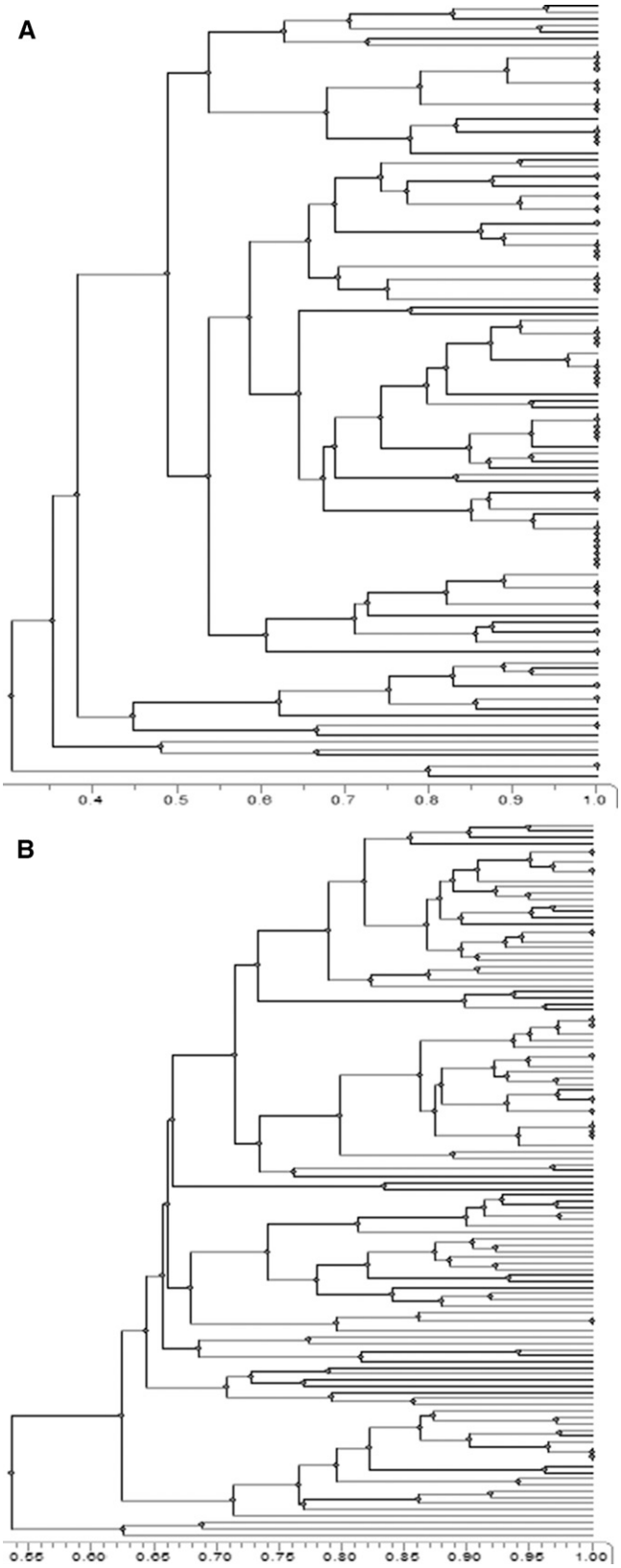

FIGURE 1. (**A**) UPMGA dendrogram of ERIC types. (**B**) UPMGA dendrogram of PFGE patterns.

system and imported into the Phoretix gel analysis program as above. Bands were normalized, and lane images were exported to a Phoretix database. A UPMGA diagram based on dice coefficients was constructed (Figure 1B), and clusters with coefficients of ≥ 85% were identified as the same pulse type.

Statistical analysis. The discriminating abilities of ERIC-PCR and PFGE were calculated by the use of Simpson's index of diversity,[15] and 95% confidence intervals (CIs) were calculated using the formula in the work by Grundmann and others.[16] The association of the presence or absence of diarrhea or dysentery with a specified serotype or molecular type as defined by PFGE or ERIC-PCR was evaluated by using an odds ratio with Mantel–Haenzel 95% CIs. In cases where a cell value was equal to zero, a χ^2 value was calculated using SPSS v 13 (Chicago, IL).

RESULTS

Antigenic typing of the isolates identified a highly diverse group of isolates with *S. flexneri* as the serogroup in 72.4% (84) of the isolates. Among the *S. flexneri* isolates, 26.7% (31) of the isolates were serotype 2a, 20.7% (24) were serotype 3a, 7.8% (9) were serotype 4a, 6.9% (8) were serotype 6, 5.2% (6) were serotype 1b, 2.6% (3) were serotype 4b, 1.7% (2) were serotype Y, and 0.9% (1) were serotype 2b. *S. sonnei* accounted for 13.8% of all isolates, and *S. boydii* and *S. dysenteriae* accounted for 11.2% (13) and 0.9% (1) of isolates, respectively. Two isolates (1.7%) did not agglutinate with the typing antisera and were classified as *Shigella* spp.

PFGE typing distinguished 37 different pattern types. The most prevalent pattern was present in 19% of all isolates,

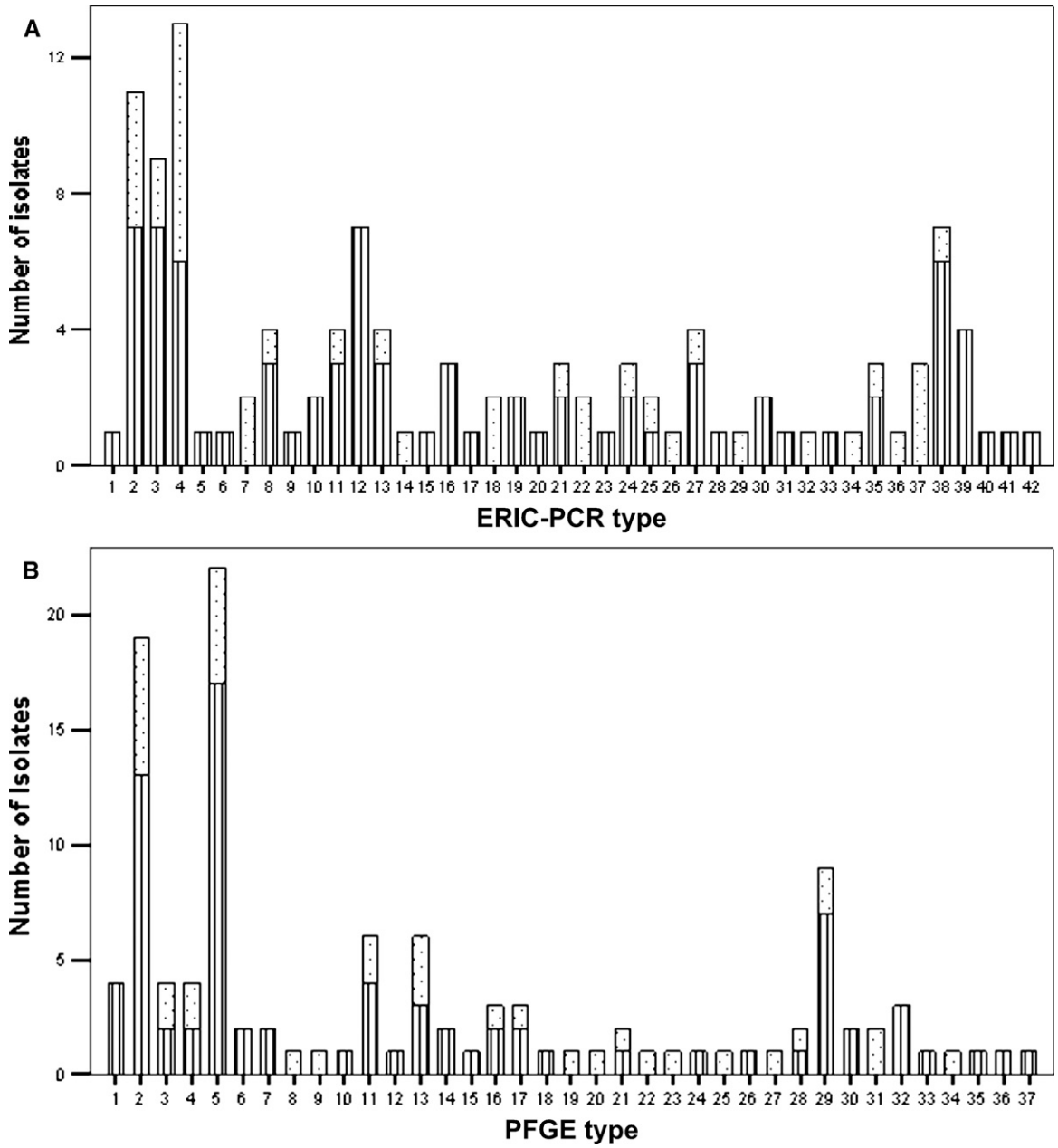

FIGURE 2. (**A**) Frequency distribution of ERIC types. (**B**) Frequency distribution of PFGE types. Dotted areas refer to isolates from asymptomatic cases, and striped areas represent isolates from patients with diarrhea.

whereas 19 restriction types were present in only one isolate each (Figure 2B). The discrimination index of PFGE was 0.925 (95% CI = 0.830–1.00). ERIC-PCR typing distinguished 42 distinct pattern types (Figure 2A). The most prevalent type described 11.2% of the isolates. Seventeen ERIC types were unique. The discrimination index of ERIC-PCR was 0.961 (95% CI = 0.886–1.00). When serogroup and serotype results were compared with molecular profiles (Table 1), it was found that 89% (33) of 37 PFGE patterns were restricted to a single serogroup. Four PFGE profiles had one pattern that belonged to a second serogroup described by the same genomic pattern; 51% (16/31) of the most common *S. flexneri* serotype, serotype 2a, were described by a single PFGE pulse type (pulse type 2), although *S. flexneri* 2a isolates were spread among six different

TABLE 1
Isolates are shown with the designated molecular typing patterns as determined by PFGE, ERIC-PCR, serogroup, and serotype

Isolate	PFGE	ERIC	Serogroup	Serotype	Isolate	PFGE	ERIC	Serogroup	Serotype
SHI1309	1	1	*S. flexneri*	2a	SHI0560	11	20	*S. flexneri*	6
SHI2493	1	2	*S. flexneri*	2a	SHI2213	11	21	*S. flexneri*	6
SHI1539	1	3	*S. flexneri*	2a	SHI2085	11	21	*S. flexneri*	6
SHI0261	1	4	*S. flexneri*	2a	SHI0079	11	22	*S. flexneri*	6
SHI2944	2	2	*S. flexneri*	2a	SHI1909	11	20	*S. flexneri*	6
SHI0602	2	4	*S. flexneri*	2a	SHI2257	11	23	*S. flexneri*	6
SHI3622	2	4	*S. flexneri*	2a	SHI5653	12	24	*S. flexneri*	6
SHI2020	2	2	*S. flexneri*	2a	SHI1797	13	18	*S. flexneri*	4a
ME180	2	3	*S. flexneri*	2a	SHI1498	13	8	*S. flexneri*	4b
SHI2144	2	2	*S. flexneri*	2a	SHI1756	13	8	*S. flexneri*	4a
SHI3000	2	2	*S. flexneri*	2a	SHI1228	13	8	*S. flexneri*	4a
ME081	2	4	*S. flexneri*	2a	SHI1321	13	25	*S. flexneri*	4a
SHI2077	2	2	*S. flexneri*	2a	SHI1936	13	26	*S. flexneri*	4a
SHI1838	2	2	*S. flexneri*	2a	SHI5555	14	27	*S. flexneri*	4a
SHI1570	2	2	*S. flexneri*	2a	SHI2102	14	27	*S. flexneri*	4a
SHI0461	2	4	*S. flexneri*	2a	SHI2659	15	27	*S. flexneri*	4a
SHI2115	2	5	*S. flexneri*	2a	ME106	16	25	*S. flexneri*	2b
SHI1234	2	2	*S. flexneri*	Y	SHI3180	16	28	*S. flexneri*	4a
SHI0356	2	4	*S. flexneri*	2a	SHI1608	16	27	*S. flexneri*	4b
SHI0146	2	4	*S. sonnei*		SHI4604	17	29	*S. boydii*	
SHI0046	2	4	*S. flexneri*	2a	SHI4056	17	30	*S. boydii*	
SHI1949	2	4	*S. flexneri*	3a	SHI4028	17	30	*S. boydii*	
SHI2162	2	2	*S. flexneri*	2a	SHI3944	18	24	*S. boydii*	
SHI4744	3	3	*S. flexneri*	2a	SHI4641	19	24	*S. boydii*	
SHI1893	3	4	*S. flexneri*	Y	SHI4023	20	22	*S. boydii*	
ME130	3	3	*S. flexneri*	2a	SHI0107	21	2	*S. flexneri*	1b
SHI3256	3	3	*S. flexneri*	2a	SHI3734	21	31	*S. flexneri*	6
SHI2370	4	6	*S. flexneri*	2a	SHI0444	22	7	*S. flexneri*	1b
SHI3605	4	4	*S. flexneri*	2a	SHI0189	23	32	*S. boydii*	
SHI3066	4	3	*S. flexneri*	2a	SHI0162	24	33	*S. species*	
SHI1858	4	7	*S. flexneri*	2a	SHI0186	25	34	*S. boydii*	
SHI4695	5	8	*S. flexneri*	1b	SHI0403	26	19	*S. flexneri*	3a
SHI4669	5	9	*S. flexneri*	3a	SHI0230	27	4	*S. boydii*	
SHI2049	5	10	*S. flexneri*	3a	SHI0285	28	35	*S. boydii*	
SHI2539	5	11	*S. flexneri*	3a	SHI4644	28	36	*S. species*	
SHI3522	5	12	*S. flexneri*	3a	SHI5539	29	37	*S. sonnei*	
SHI4183	5	13	*S. flexneri*	1b	SHI3493	29	38	*S. sonnei*	
SHI4066	5	14	*S. flexneri*	2a	SHI0221	29	38	*S. sonnei*	
ME132	5	10	*S. flexneri*	3a	SHI1211	29	39	*S. sonnei*	
SHI0423	5	12	*S. flexneri*	3a	SHI3152	29	38	*S. sonnei*	
SHI2039	5	15	*S. flexneri*	3a	SHI3168	29	39	*S. sonnei*	
SHI2975	5	13	*S. flexneri*	3a	SHI2907	29	39	*S. sonnei*	
ME110	5	13	*S. flexneri*	3a	SHI5710	29	3	*S. flexneri*	2a
SHI2770	5	13	*S. flexneri*	3a	SHI1918	29	40	*S. sonnei*	
SHI3169	5	16	*S. flexneri*	3a	SHI1866	30	38	*S. sonnei*	
ME115	5	12	*S. flexneri*	3a	SHI3190	30	39	*S. sonnei*	
ME131	5	17	*S. flexneri*	3a	SHI0267	31	35	*S. boydii*	
ME114	5	16	*S. flexneri*	3a	SHI5538	31	37	*S. sonnei*	
SHI1958	5	18	*S. flexneri*	3a	SHI3243	32	38	*S. sonnei*	
SHI3083	5	11	*S. flexneri*	3a	SHI1614	32	38	*S. sonnei*	
ME121	5	12	*S. flexneri*	1b	ME134	32	16	*S. sonnei*	
ME133	5	12	*S. flexneri*	3a	SHI0994	33	38	*S. sonnei*	
SHI2973	5	12	*S. flexneri*	3a	SHI3590	34	37	*S. boydii*	
SHI2618	6	11	*S. flexneri*	3a	SHI2946	35	35	*S. dysentariae*	
SHI3114	6	12	*S. flexneri*	3a	SHI2950	36	40	*S. boydii*	
SHI1867	7	19	*S. flexneri*	3a	SHI1484	37	42	*S. flexneri*	4b
SHI2305	7	11	*S. flexneri*	3a					
SHI4899	8	3	*S. flexneri*	1b					
SHI3002	9	4	*S. flexneri*	2a					
SHI4556	10	3	*S. flexneri*	2a					

TABLE 2
Pairwise comparison of typing strategies for ERIC-PCR and PFGE

	PFGE	
	C	NC
ERIC-PCR		
C	150	363
NC	277	5,880

C = clonal; NC = non-clonal.

pulse types (1, 2, 3, 4, 5, 9, and 29). *S. flexneri* 3a, the second most common serotype, had 75% (18/24) of isolates fall into the same pulse type (pulse type 5), although 3a isolates fell into five different pulse types (2, 5, 6, 7, and 26) . ERIC types were restricted to serogroups in 86% (36/42) of cases. However, ERIC types distinguished more molecular types within common serotypes. In the case of *S. flexneri*, 2a isolates were divided into nine ERIC types (1–7, 9, and 40) but in principle, into three different ERIC patterns, whereas no more than nine isolates (29%) fell into one ERIC pattern. Similarly, among *S. flexneri* 3a isolates, no more than 25% of this serotype (six isolates) fell into one ERIC pattern.

Because the primary intent of either genotyping strategy is to determine if an isolate is clonal or non-clonal compared with a reference, pairwise comparisons of dice coefficients between the 116 isolates (6,670 permutations) were made at dice coefficients of 80% and 85% in turn to determine the concordance in determining clonality between the two tests. The observed agreement between the two tests was 90.4% at the 85% level, which fell to 85.1% at the 80% level. The assignment of clonal versus non-clonal between the two typing strategies was highly correlated (Pearson $\chi 2$ $P < 0.000$). Contingency tables comparing the assignment of

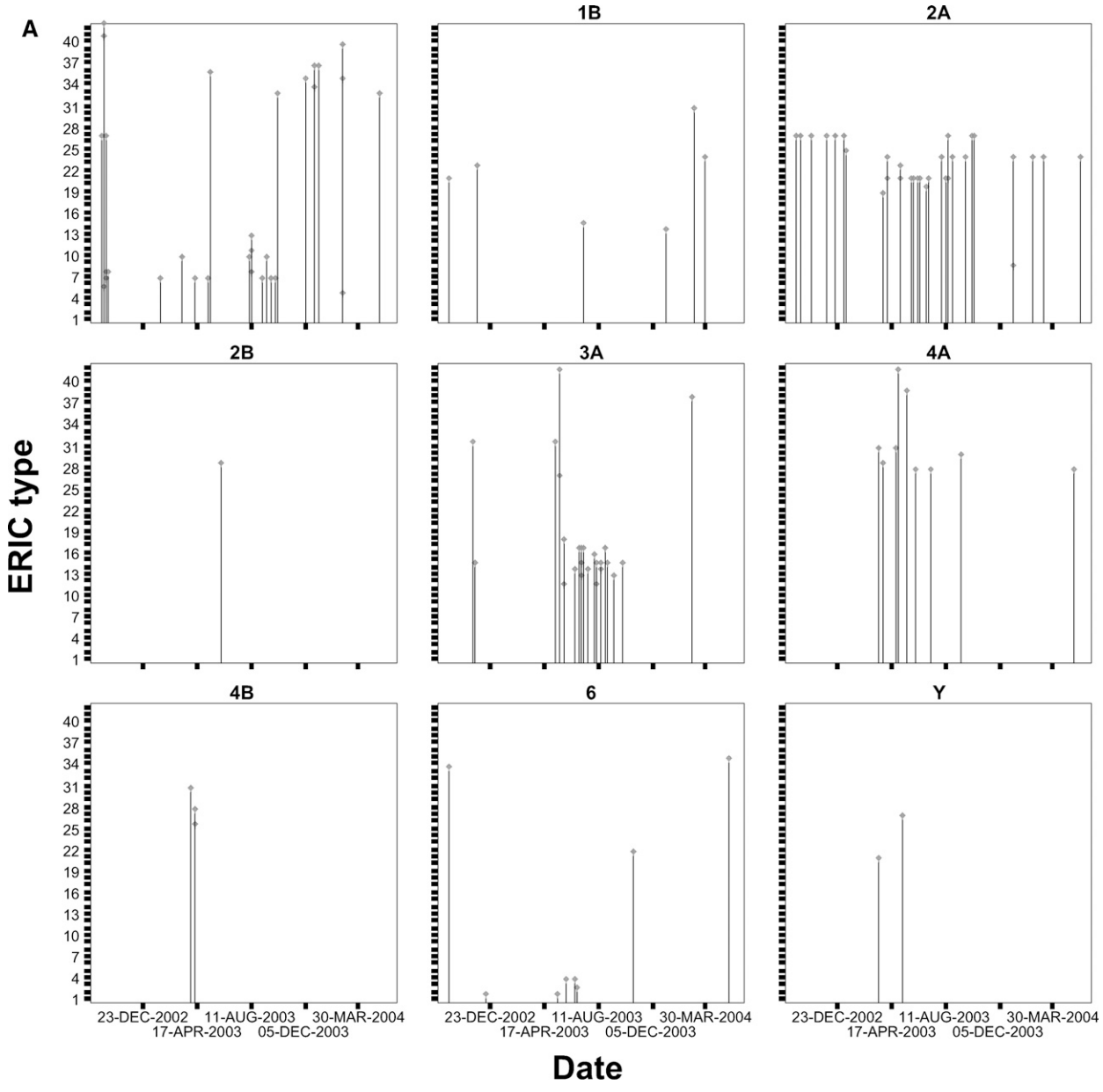

FIGURE 3. (**A**) ERIC patterns over the study period grouped by serotype (*S. flexneri* only; the rest appear in a single graphic).

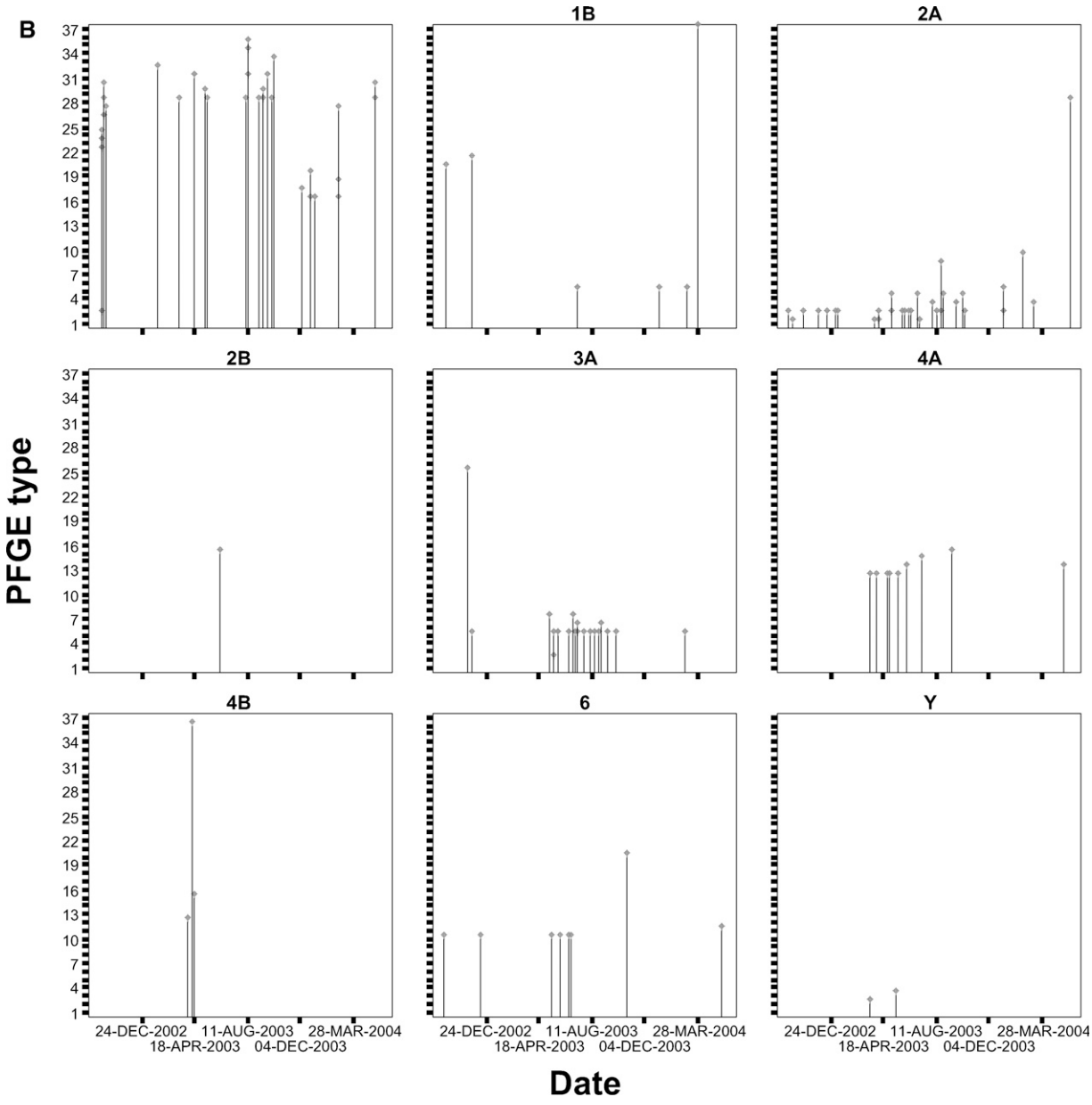

FIGURE 3. (**B**) PFGE pulse types over the study period grouped by serotype (*S. flexneri* only; the rest appear in a single graphic).

paired comparisons of ERIC with PFGE are shown in Table 2. If one assumes that PFGE is the standard for typing *Shigella* isolates, then the sensitivity (in detecting a clonal relationship) is 35.1% (95% CI = 30.8–39.8), the specificity of ERIC-PCR is 94.2% (95% CI = 93.6–94.7), the positive predictive value of the identification of a pair as clonal is 29.2% (95% CI = 25.5–33.3), and the negative predictive value is 95.5% (95% CI = 95.0–96.0). Isolates were highly diverse, which was shown by both molecular typing strategies; between 80% and 88% of the pairwise comparisons were described as non-clonal by both ERIC and PFGE, and the dice coefficient relating the most distant clusters was only 50%.

To compare the ability of ERIC-PCR and PFGE to describe the dynamics of transmission of *Shigella* beyond serotyping alone, scatterplots were generated of molecular types over time and subgrouped by *S. flexneri* serotype (Figure 3). Interpretation of the ERIC-PCR types and pulse types led to similar conclusions over time, both of which enriched knowledge of disease transmission relative to the use of serotype alone. For example, the initial *S. flexneri* 2a strain circulating in this community between September of 2002 and December of 2002 was restricted to two molecular types that were closely related. Between April and December of 2003, both molecular typing methods revealed five relatively closely related strains in circulation in the community. A cluster of three *Shigella flexneri* 4a cases in April of 2003 were not closely related by either ERIC-PCR or PFGE typing. The cluster of *S. flexneri* 3a cases between April and September of 2003 was caused by a group of closely related molecular types as indicated by typing from both methodologies.

Of the 116 isolates, 68% (79) were from children with diarrhea, and 32% (37) were asymptomatic. Of the individuals

with *Shigella* and diarrhea, 28% (22) had dysenteric diarrhea (defined as visible blood in an unformed stool in a patient reporting over three unformed evacuation in the last 24 hours), and the remaining 72% (57) had non-dysenteric diarrhea. No single ERIC-PCR or PFGE type was significantly associated with the development of diarrhea or dysentery in part because of the large number of types and limited sample size. Compared with other types of shigellosis, it was found that individuals infected with *S. flexneri* 3a had over six times the risk of developing diarrhea (odds ratio = 6.75, 95% CI = 1.4–44.3) as patients infected with other serotypes of Shigella. Infection with *S. flexneri* 1b, however, was rarely associated with diarrhea compared with other serotypes of Shigella (odds ratio = 0.08, 95% CI = 0–0.77).

DISCUSSION

This comparative study of molecular typing methods for Shigella revealed that ERIC-PCR is a plausible alternative strategy to PFGE. The simplicity, ease of operation, low per isolate cost, and limited need for specialized laboratory equipment favor its use over PFGE. Our evaluation of an expanded number and diversity of Shigella isolates has confirmed the use of ERIC-PCR that the work by Liu and others[11] described in the typing of 20 *S. sonnei* isolates.

It should be noted that our findings are in contrast to the findings in the work by Surdeanu and others,[17] which also compared PFGE with ERIC-PCR and found significantly worse test performance of ERIC-PCR compared with PFGE. In their study of 97 *S. flexneri* strains, they described 31 pulse types but only seven ERIC-PCR patterns. Several differences in specimen processing and analysis may explain the differences in our results. The first difference is the use of a different ERIC primer. In early work, we found that ERIC 1 alone, as used in the work by Liu and others[11], yielded more complex band patterns than ERIC 2 (as used in the work by Surdeanu and others[17]) or a combination of ERIC 1 and ERIC 2 (data not shown). Additionally, although pulse types for PFGE were analyzed by dice coefficients in the work by Surdeanu and others,[17] ERIC patterns were visually compared, and the decision to call isolates identical or nonidentical was on the basis of similar numbers and positions of major bands. The number of patterns analyzed significantly exceeds recommendations for visual band analysis.[18] Bands classified as faint were ignored in their comparative analysis. Obviating visible bands during a comparative analysis may lead to oversimplification of band patterns, especially in typing techniques where the number of bands per pattern is relatively low (ERIC-PCR and RAPD-PCR). Additionally, we found that both repeatability and complexity of patterns were enhanced by DNA quantification before the assay, a step that is commonly part of PFGE and RAPD protocols but not described in ERIC protocols.

ERIC sequences are short (127 bp), imperfect palindromic sequences present in Enterobacteracaea as well as *Vibrio cholerae*. Recent genomic work on ERIC sequences in *Escherichia coli* and Shigella revealed that *S. flexneri* 2a 301 has 15 full-length and 11 partial-length ERIC sequences, *S. dysenteriae* Sd197 has 12 full-length and 8 partial-length copies, *S. sonnei Ss046* has 18 full-length copies and 11 partial copies, and *S. boydii* Sb227 has 19 full-length copies and 10 partial-length copies.[19] All copies were chromosomal. The spacing of sequences and the improbable hybridization in some partial sequences suggest that band products visualized from typing are not caused by exclusive hybridization to ERIC sequences, and the primers, therefore, function, at least to some extent, as low-stringency arbitrary primers. The ERIC-PCR method is highly similar to, if not always, a RAPD assay. The longer length of the ERIC-PCR (22 bp) compared with 10–12 bp in standard RAPD primers seems to increase the efficiency of primers and the robustness of the assay compared with RAPDs.[20]

Although a national and perhaps international standardized typing protocol and database, such as the database used by PulseNet, has an unquestionable value in determining the movement of strains over wide geographic areas, other typing methods, such as ERIC-PCR, also may have important complementary roles. ERIC-PCR yields final analyzable images 8 hours after the extraction of DNA. The PulseNet 12-hour protocol does not yield final results for 36 hours, because the gel runs for 22 hours after the initial 12 hours needed for lysis, washes, and enzyme digestion. The cost of ERIC-PCR is $8 per isolate, which compares well with the cost of PFGE that is commercially offered at $75.[21] Standard gels and electrophoresis units used for PCR-ERIC allow for more specimens to be concurrently evaluated. The technique requires no specialized equipment or reagents in a laboratory with PCR capabilities. Because the number of organisms that are successfully typed with ERIC-PCR continues to grow to include diarrheogenic[22] and uropathogenic *E. coli*,[23] *Pseudomonas aeruginosa*,[24] *Aeromonas hydrophila*,[25] *Vibrio parahemolyticus*,[26] Stenotrophomonas,[24] *Klebsiella pneumoniae*,[27] *Yersinia enterocolitica*,[28] *Bartonella henselea*,[29] and *Helicobacter pylori*,[20] the advantage of adopting this technique at the hospital and regional level also increases. Experience with this simplified technique allows for the preliminary investigation of many important nosocomial or regional outbreaks without the need for a disease-specific approach after primary culture. This molecular typing may be done in parallel or independently from serotyping of the isolates under investigation to yield important information on the clonality of isolates. ERIC reagents are affordable, standard, and have the relative advantage over antisera of long-term stability and affordability. The relative advantage for this test in limited resource settings where shigellosis is endemic is significantly greater than in the United States and Europe, where PFGE analysis is not likely to be facilitated by government-funded agencies. Although grouping serum may be available at the level of national laboratories, complete typing kits from Denka-Seiken include a kit of 43 antisera required for the full typing of isolates. At any one time, a few serotypes are likely to predominate, and in the case of *S. sonnei*, which contains only a single serotype, ERIC-PCR offers a rapid way to analyze temporally associated isolates to determine transmission patterns.

Received October 28, 2011. Accepted for publication March 3, 2012.

Acknowledgments: The study was funded by National Institutes of Health Fogarty Grant K01TW5717 (to M.K.). The authors would like to thank Hugo Rodriguez from the Health Ministry of Loreto for collaboration and S. T. Unt for critical review of the manuscript. The views expressed in this article are the views of the authors and do not

necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, or the US Government.

Authors' addresses: Margaret Kosek, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, E-mail: mkosek@jhsph.edu. Pablo Peñataro Yori, Universidad Peruana Cayetano Heredia, Laboratorio de Investigacion y Desarrollo, Lima, Peru, E-mail: pyori@jhsph.edu. Robert H. Gilman, Johns Hopkins Bloomberg School of Public Health, International Health, Baltimore, MD, E-mail: rgilman@jhsph.edu. Henry Vela and Maritza Calderon, Universidad Peruana Cayetano Herediam, Laboratorio de Investigacion y Desarrollo, Lima, Peru, E-mails: hjvela@gmail.com and mcalderon@yahoo.es. Maribel Paredes Olortegui and Cesar Banda Chavez, Asociacion Benefica PRISMA, Investigaciones Biomedicas, Iquitos, Peru, E-mails: mparedeso@prisma.org.pe and cbandac@prisma.org.pe. Juan Perez Bao, Naval Medical Research Unit 6, Biostatistics, Lima, Peru, E-mail: juan.perez@med.navy.mil. Eric Hall, Ryan Maves, and Rosa Burga, Naval Medical Research Unit 6, Microbiology, Lima, Peru, E-mails: eric.hall@med.navy.mil, ryan.maves@med.navy.mil, and rosaburga@gmail.com. Graciela Meza Sanchez, Ministry of Health, Loreto, Villa Punchana, Peru, E-mail: gracielamezasanchez@yahoo.com.

REFERENCES

1. Riddle MS, Sanders JW, Putnam SD, Tribble DR, 2006. Incidence, etiology, and impact of diarrhea among long-term travelers (US military and similar populations): a systematic review. *Am J Trop Med Hyg 74:* 891–900.
2. Agtini MD, Soeharno R, Lesmana M, Punjabi NH, Simanjuntak C, Wangsasaputra F, Nurdin D, Pulungsih SP, Rofiq A, Santoso H, Pujarwoto H, Sjahrurachman A, Sudarmono P, Von Seidlein L, Deen JL, Ali M, Lee H, Kim DR, Han O, Park JK, Suwandono A, Ingerani, Oyofo BA, Campbell JR, Beecham HJ, Corwin AL, Clemens JD, 2005. The burden of diarrhoea, shigellosis, and cholera in North Jakarta, Indonesia: findings from 24 months surveillance. *BMC Infect Dis 5:* 89–100.
3. Jones FR, Sanchez JL, Meza R, Batsel TM, Burga R, Canal E, Block K, Perez J, Bautista CT, Escobedo J, Walz SE, 2004. Short report: high incidence of shigellosis among Peruvian soldiers deployed in the Amazon River basin. *Am J Trop Med Hyg 70:* 663–665.
4. Thornton SA, Sherman SS, Farkas T, Zhong W, Torres P, Jiang X, 2005. Gastroenteritis in US Marines during Operation Iraqi Freedom. *Clin Infect Dis 40:* 519–525.
5. Kolavic SA, Kimura A, Simons SL, Slutsker L, Barth S, Haley CE, 1997. An outbreak of *Shigella dysenteriae* type 2 among laboratory workers due to intentional food contamination. *JAMA 278:* 396–398.
6. Anon, 1995. Public health impact of Rwandan refugee crisis: what happened in Goma, Zaire, in July, 1994? Goma Epidemiology Group. *Lancet 345:* 339–344.
7. Buysse JM, Hartman AB, Strockbine N, Venkatesan M, 1995. Genetic polymorphism of the ipaH multicopy antigen gene in *Shigella* spps. and enteroinvasive *Escherichia coli. Microb Pathog 19:* 335–349.
8. Karaolis DK, Lan R, Reeves PR, 1994. Sequence variation in *Shigella sonnei* (Sonnei), a pathogenic clone of *Escherichia coli*, over four continents and 41 years. *J Clin Microbiol 32:* 796–802.
9. Soldati L, Piffaretti JC, 1991. Molecular typing of Shigella strains using pulsed field gel electrophoresis and genome hybridization with insertion sequences. *Res Microbiol 142:* 489–498.
10. Coimbra RS, Grimont F, Grimont PA, 1999. Identification of Shigella serotypes by restriction of amplified O-antigen gene cluster. *Res Microbiol 150:* 543–553.
11. Liu PY, Lau YJ, Hu BS, Shyr JM, Shi ZY, Tsai WS, Lin YH, Tseng CY, 1995. Analysis of clonal relationships among isolates of *Shigella sonnei* by different molecular typing methods. *J Clin Microbiol 33:* 1779–1783.
12. Navia MM, Capitano L, Ruiz J, Vargas M, Urassa H, Schellemberg D, Gascon J, Vila J, 1999. Typing and characterization of mechanisms of resistance of *Shigella* spp. isolated from feces of children under 5 years of age from Ifakara, Tanzania. *J Clin Microbiol 37:* 3113–3117.
13. Bando SY, do Valle GR, Martinez MB, Trabulsi LR, Moreira-Filho CA, 1998. Characterization of enteroinvasive *Escherichia coli* and Shigella strains by RAPD analysis. *FEMS Microbiol Lett 165:* 159–165.
14. Kosek M, Yori PP, Pan WK, Olortegui MP, Gilman RH, Perez J, Chavez CB, Sanchez GM, Burga R, Hall E, 2008. Epidemiology of highly endemic multiply antibiotic-resistant shigellosis in children in the Peruvian Amazon. *Pediatrics 122:* e541–e549.
15. Hunter PR, Gaston MA, 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J Clin Microbiol 26:* 2465–2466.
16. Grundmann H, Hori S, Tanner G, 2001. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. *J Clin Microbiol 39:* 4190–4192.
17. Surdeanu M, Ciudin L, Pencu E, Straut M, 2003. Comparative study of three different DNA fingerprint techniques for molecular typing of *Shigella flexneri* strains isolated in Romania. *Eur J Epidemiol 18:* 703–710.
18. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B, 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol 33:* 2233–2239.
19. Wilson LA, Sharp PM, 2006. Enterobacterial repetitive intergenic consensus (ERIC) sequences in *Escherichia coli*: Evolution and implications for ERIC-PCR. *Mol Biol Evol 23:* 1156–1168.
20. Finger SA, Velapatino B, Kosek M, Santivanez L, Dailidiene D, Quino W, Balqui J, Herrera P, Berg DE, Gilman RH, 2006. Effectiveness of enterobacterial repetitive intergenic consensus PCR and random amplified polymorphic DNA fingerprinting for *Helicobacter pylori* strain differentiation. *Appl Environ Microbiol 72:* 4713–4716.
21. Drinka PJ, Stemper ME, Gauerke CD, Miller JM, Reed KD, 2004. The identification of genetically related bacterial isolates using pulsed field gel electrophoresis on nursing home units: a clinical experience. *J Am Geriatr Soc 52:* 1373–1377.
22. Dalla-Costa LM, Irino K, Rodrigues J, Rivera IN, Trabulsi LR, 1998. Characterisation of diarrhoeagenic *Escherichia coli* clones by ribotyping and ERIC-PCR. *J Med Microbiol 47:* 227–234.
23. Manges AR, Natarajan P, Solberg OD, Dietrich PS, Riley LW, 2006. The changing prevalence of drug-resistant *Escherichia coli* clonal groups in a community: evidence for community outbreaks of urinary tract infections. *Epidemiol Infect 134:* 425–431.
24. Silbert S, Pfaller MA, Hollis RJ, Barth AL, Sader HS, 2004. Evaluation of three molecular typing techniques for nonfermentative Gram-negative bacilli. *Infect Control Hosp Epidemiol 25:* 847–851.
25. Davin-Regli A, Bollet C, Chamorey E, Colonna D'istria V, Cremieux A, 1998. A cluster of cases of infections due to *Aeromonas hydrophila* revealed by combined RAPD and ERIC-PCR. *J Med Microbiol 47:* 499–504.
26. Wong HC, Lin CH, 2001. Evaluation of typing of vibrio parahaemolyticus by three PCR methods using specific primers. *J Clin Microbiol 39:* 4233–4240.
27. Gazouli M, Kaufmann ME, Tzelepi E, Dimopoulou H, Paniara O, Tzouvelekis LS, 1997. Study of an outbreak of cefoxitin-resistant *Klebsiella pneumoniae* in a general hospital. *J Clin Microbiol 35:* 508–510.
28. Souza RA, Pitondo-Silva A, Falcao DP, Falcao JP, 2010. Evaluation of four molecular typing methodologies as tools for determining taxonomy relations and for identifying species among Yersinia isolates. *J Microbiol Methods 82:* 141–150.
29. Sander A, Ruess M, Bereswill S, Schuppler M, Steinbrueckner B, 1998. Comparison of different DNA fingerprinting techniques for molecular typing of Bartonella henselae isolates. *J Clin Microbiol 36:* 2973–2981.