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Title: Antimicrobial sensitivity trends and virulence genes in *Shigella* spp. from the Oceania region

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Abstract

Shigella is a common cause of diarrhoea in Papua New Guinea (PNG) and other Oceania countries. However, little is known about the strains causing infection. Archived *Shigella* isolates (n=72) were obtained from research laboratories in PNG and reference laboratories in Australia. *Shigella* virulence genes were detected by PCR, and antimicrobial susceptibility was determined by disk diffusion. The *ipaH* virulence gene was present in all 72 isolates. The prevalence of other virulence genes was variable, with *ial*, *invE*, *ipaBCD*, *sen/ospD3* and *virF* present in 60% of isolates and *set1A* and *set1B* genes present in 42% of isolates. Most *S. flexneri* isolates contained genes encoding enterotoxin 1 and/ or enterotoxin 2. Resistance to antibiotics was common, with 51/72 isolates resistant to 2-4 antimicrobials. A greater proportion of bacteria isolated since 2010 (relative to pre-2010 isolates) were resistant to commonly used antibiotics such as ampicillin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole; suggesting that antimicrobial resistance (AMR) in *Shigella* is increasing over time in the Oceania region. There is a need for improved knowledge regarding *Shigella* circulation in the Oceania region and further monitoring of AMR patterns.

Abbreviations:

AMR: antimicrobial resistance

DNA: Deoxyribonucleic acid

MDR: multi-drug resistant

PCR: polymerase chain reaction

PNG: Papua New Guinea

Keywords:

Shigellosis, *Shigella*, antimicrobial resistance, virulence genes, surveillance, Oceania, Papua New Guinea

Main text

Shigellosis is a leading global cause of moderate-severe diarrhoea in children (Bardhan et al. 2010) and adults (Lozano et al. 2012), affecting ~125 million people per year. There are four species of *Shigella*, each with their own epidemiological characteristics. *S. flexneri* is responsible for most of the shigellosis burden in developing countries globally; with *S. boydii* also important, but largely confined to the Indian subcontinent. *S. dysenteriae* causes sporadic, epidemic outbreaks of diarrhoea, especially in areas experiencing conflict and natural disasters (Kotloff et al. 1999). *S. sonnei* occurs predominantly in developed countries and in countries transitioning from low- to middle-income (Niyogi 2005; Taneja and Mewara 2016).

Papua New Guinea (PNG) is the only low/middle income country in the Oceania region for which recent reports on shigellosis exist, with evidence that *Shigella* is an important cause of enteric disease in adults (Greenhill et al. 2014), children (Howard et al. 2000; Soli et al. 2014) and vulnerable populations (Benny et al. 2014). Little is

known about the genetic traits or virulence of *Shigella* strains circulating in the Oceania region, beyond the sporadic documentation of antibiotic resistance to many commonly used antibiotics such as ampicillin, chloramphenicol, tetracycline and trimethoprim-sulfamethoxazole (Greenhill et al. 2014; Rosewell et al. 2010; Storch et al. 1980; Watson 2006). In this study, we sought to gain a greater understanding of *Shigella* isolated from PNG and nearby Oceania countries, looking specifically at the presence of virulence genes and antibiotic resistance.

Seventy-two *Shigella* isolates from PNG (n=60) and neighbouring Pacific Island nations (n=12) were analysed. Of the PNG isolates: 30 were from a study previously conducted by our research team (Greenhill et al. 2014); one was from a recent outbreak of shigellosis in PNG (Benny et al. 2014); seven isolates were from a previous case-control study conducted in PNG (Howard et al. 2000); and the remaining isolates (n = 22) were from travellers returning to Australia from PNG. The 12 non-PNG isolates were from travellers returning to Australia from Pacific Island nations (Fiji, Vanuatu, Samoa, Solomon Islands), including three isolates from people who lived in or had spent time in the Torres Strait Islands (situated north of mainland Australia). The study collection comprised 53 *S. flexneri* isolates (from 1985-2014), 16 *S. sonnei* isolates (from 1999-2015) and three *S. dysenteriae* isolates (from 1985 and 2010).

Isolates were cultured on nutrient agar and incubated overnight at 37°C prior to DNA extraction (FavorPrep Tissue Genomic DNA Extraction Mini Kit, Favorgen, Taiwan), and antibiotic susceptibility testing. Isolates had previously been confirmed as *Shigella* and speciated using poly-O antisera; all 72 isolates were confirmed as

Shigella by real-time PCR detection of the *ipaH* gene (Lin et al. 2010) prior to further analyses. Approximately half (56%) the isolates were serotyped/biotyped.

Conventional PCR (Table 1) was used to confirm the presence/absence of known *Shigella* virulence genes: *ipaBCD* and *ipaH* (invasion plasmid antigen BCD and H genes); *ial* (invasion-associated locus); *virF* and *invE* (regulators for transcription activation on the virulence plasmid); *set1A* and *set1B* (*Shigella* enterotoxin 1 genes); and *sen/ospD3* (*Shigella* enterotoxin 2 gene). Amplicons were visualised by gel electrophoresis.

Isolates were tested for antibiotic susceptibility by disk diffusion following the Clinical Laboratory Standards Institute guidelines (CLSI 2015), using *Escherichia coli* ATCC 25922 as a control. Antibiotics assayed were ampicillin (10 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), tetracycline (30 µg) and trimethoprim-sulfamethoxazole (25 µg).

Isolates of each species were grouped according to their virulence gene profiles (presence/absence of genes), producing four groups of *S. flexneri*, two groups of *S. sonnei* and a single group of *S. dysenteriae* (Table 2). Detailed statistical analyses were not conducted due to opportunistic sample collection, but a trend for more recent *S. flexneri* isolates to harbour more virulence genes was noted. In particular, genes coding for *Shigella* enterotoxin 1 (*set1A* and *set1B*) were detected only in isolates obtained since 2009. All isolates positive for *set1A* and *set1B* were from PNG, or from the Torres Strait Islands - a region of Australia with close geographical and cultural links to PNG.

The enterotoxin genes are primarily responsible for the clinical manifestation of acute-watery diarrhoea and dysentery in shigellosis. Genes encoding *Shigella* enterotoxin 1 (*set1A* and *set1B*) were present in *S. flexneri* serotype 2a isolates collected from 2009 onwards (~57% of *S. flexneri* isolates) but not detected in either *S. sonnei* or *S. dysenteriae*. These genes have been detected at similar rates in paediatric cases in the Brazilian Amazon (da Cruz et al. 2014) and in northeastern Brazil (Mendeiros et al, 2018), but at lower rates in comparable studies in Argentina (Casabonne et al. 2016). No published studies have investigated virulence genes in *Shigella* from the Oceania region, and relatively few in Asia. In China a similar species distribution of the s*et* gene was observed in Beijing, being commonly detected in *S. flexneri* (88% of isolates, thus more frequently than in PNG isolates), but infrequently in other species (Qu et al, 2014). In a study conducted in Eastern China *set-1A* and *set-1B* genes were detected in 74.5% and 78% of *S. flexneri*, respectively (Fan et al, 2017) In our study *Shigella* enterotoxin 2 genes (*sen/ospD3*) were present in 79% of *S. flexneri* isolates, a detection rate higher than two comparable studies conducted in South America (da Cruz et al. 2014; Casabonne et al. 2016) but lower than detected in *S. flexneri* isolates from northeastern Brazil (Medeiros et al, 2018). One isolate of *S. sonnei* harboured *sen/ospD3*, which has been reported previously (da Cruz et al. 2014; Casabonne et al. 2016); and indeed appears common in parts of Brazil (Medeiros et al, 2018) and China (Qu et al, 2014; Fan et al, 2017). The high prevalence of both enterotoxin encoding genes in circulating strains of *S. flexneri* could have public health ramifications in PNG, where access to healthcare is often limited.

The *Shigella* invasion plasmid is a large unstable plasmid that encodes several virulence genes, including *ipaH*, *ipaBCD*, *virF*, *invE* and *ial*; the *ipaH* gene is also chromosomally encoded, so is considered a stable target gene. The *ipaH* gene was present in all isolates, while 60% of isolates contained all four *ipaBCD*, *virF*, *invE* and *ial* genes (Table 2). It is important to note that the latter virulence genes *(ipaBCD*, *virF*, *invE* and *ial)* all contribute to facilitating and maintaining the spread and survival of *Shigella* during infection (Broach et al, 2012; Lluque et al. 2015; Schroeder et al, 2008). Our results suggest that there is a strong association between all of these genes, as they were invariably present/absent as a set (Table 2). In regards to the stability of these invasion plasmid virulence genes, some studies have shown that the *ial* gene in particular is prone to deletion (Zhang et al, 2013).

While recently circulating isolates tended to have an increased number of virulence genes, *S. flexneri* isolates with different virulence gene profiles were isolated as recently as 2012-14, suggesting co-circulation of strains. A study of global isolates of *S. flexneri* using whole genome sequencing revealed old and new strains of *S. flexneri* to persist alongside one another and continue causing infection (Connor et al. 2015).

S. flexneri isolates were frequently resistant to ampicillin (77%), tetracycline (74%), chloramphenicol (60%) and trimethoprim-sulfamethoxazole (49%). *S. sonnei* resistance to trimethoprim-sulfamethoxazole (75%) and ampicillin (56%) was common, followed by tetracycline (19%) and nalidixic acid (6%). *S. dysenteriae* also displayed resistance to ampicillin, tetracycline and trimethoprim-sulfamethoxazole (33%) (Table 3).

An increase in the prevalence of antimicrobial drug resistance to older antimicrobials, namely ampicillin, tetracycline, chloramphenicol and trimethoprim-sulfamethoxazole (Figure 1), was observed in post-2010 isolates compared to pre-2010 isolates. Few comparable data exist in the Oceania region, but this increase in antimicrobial drug resistance coincides with findings in Fiji, where there is evidence of ampicillin and chloramphenicol AMR rates increasing over time, especially in *S. flexneri* (Watson 2006). Our observed trend of increasing AMR in *Shigella* is also reflected in other low-income settings. In Mozambique, *Shigella* isolates are commonly resistant to the same antibiotics to which resistance was observed in this study (Mandomando et al, 2009). Multidrug resistance (MDR), defined as resistance to at least two antimicrobials, was detected in 71% (n=51) of isolates. Using a more stringent definition (resistance to at least three antimicrobials), MDR was detected in 54% (n=39) of isolates (Table 4); a rate also similar to that observed in Mozambique (Mandomando et al, 2009).

Resistance to ciprofloxacin or ceftriaxone was not observed in any *Shigella* isolates in this study; however, one *S. sonnei* isolate was resistant to nalidixic acid. Ciprofloxacin resistance in *S. flexneri* strains and nalidixic acid resistance in *S. sonnei* is increasingly reported in Asia and Africa (Gu et al. 2012). Given the close proximity of Asia to Oceania, the possibility of AMR strains being introduced into the Oceania region is a cause for concern and warrants monitoring.

This study provides initial data on the distribution of *Shigella* virulence genes in PNG and neighbouring Pacific Islands and provides insight into the AMR trends. It is

notable that many of the recently isolated *S. flexneri* harbour more virulence genes than most pre-2009 isolates. Similarly, there appears to be increasing resistance to commonly used antibiotics. Further genetic typing is required to determine whether these represent new strains; or they are the same strains which have acquired genetic elements associated with virulence and antibiotic resistance. There is a need to monitor *Shigella* strains in the region to enable better management of clinical cases and to reduce morbidity and mortality in vulnerable populations of the Oceania region.

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References

Bardhan, P., Faruque, A.S.G., Naheed, A., Sack, D.A., 2010. Decrease in shigellosis-related deaths without Shigella spp.-specific interventions, Asia. *Emerg Infect Dis.* 16, 1718-23.

Benny, E., Mesere, K., Pavlin, B. I., Yakam, L., Ford, R., Yoannes, M., Kisa, D., Abdad, M. Y., Menda, L., Greenhill, A. R., Horwood, P. F., 2014. A large outbreak of shigellosis commencing in an internally displaced population, Papua New Guinea, 2013. *Western Pac Surveill Response J*. 5, 18-21.

Broach, W. H., Egan, N., Wing, H. J., Payne, S. M., Murphy, E. R., 2012. VirF-independent regulation of Shigella virB transcription is mediated by the small RNA RyhB. *PLoS One*. 7(6), e38592.

Casabonne, C., Gonzalez, A., Aquili, V., Balague, C., 2016. Prevalence and Virulence Genes of Shigella spp. Isolated from Patients with Diarrhea in Rosario, Argentina. *Jpn J Infect Dis*. 69, 477-81.

CLSI., 2015. *Performance Standards for Antimicrobial Susceptibility Testing: 25th Informational Supplement. CLSI Document M100-S25.* Clinical and Laboratory Standards Institute: Wayne, USA.

Connor, T. R., Barker, C. R., Baker, K. S., Weill, F. X., Talukder, K. A., Smith, A. M., Baker, S., Gouali, M., Pham Thanh, D., Jahan Azmi, I., Dias da Silveira, W., Semmler, T., Wieler, L. H., Jenkins, C., Cravioto, A., Faruque, S. M., Parkhill, J., Wook Kim, D., Keddy, K. H.,Thomson, N. R., 2015. Species-wide whole genome sequencing reveals historical global spread and recent local persistence in Shigella flexneri. *Elife*. 4, e07335.

da Cruz, C. B., de Souza, M. C., Serra, P. T., Santos, I., Balieiro, A., Pieri, F. A., Nogueira, P. A., Orlandi, P. P., 2014. Virulence factors associated with pediatric shigellosis in Brazilian Amazon. *Biomed Res Int*., 539697.

Fan, W., Qian, H., Shang, W., Ying, C., Zhang, X., Cheng, S., Gu, B., Ma, P., 2017. Low distribution of genes encoding virulence factors in *Shigella flexneri* serotypes 1b clinical isolates from eastern Chinese populations. *Gut Pathog.* 9, 76.

Greenhill, A. R., Guwada, C., Siba, V., Michael, A., Yoannes, M., Wawarie, Y., Ford, R., Siba, P. M., Horwood, P. F., 2014. Antibiotic resistant Shigella is a

major cause of diarrhoea in the Highlands of Papua New Guinea. *J Infect Dev Ctries*. 8, 1391-7.

Gu, B., Cao, Y., Pan, S., Zhuang, L., Yu, R., Peng, Z., Qian, H., Wei, Y., Zhao, L., Liu, G., Tong, M., 2012. Comparison of the prevalence and changing resistance to nalidixic acid and ciprofloxacin of Shigella between Europe-America and Asia-Africa from 1998 to 2009. *Int J Antimicrob Agents*. 40, 9-17.

Howard, P., Alexander, N. D., Atkinson, A., Clegg, A. O., Gerega, G., Javati, A., Kajoi, M., Lupiwa, S., Lupiwa, T., Mens, M., Saleu, G., Sanders, R. C., West, B., Alpers, M. P., 2000. Bacterial, viral and parasitic aetiology of paediatric diarrhoea in the highlands of Papua New Guinea. *J Trop Pediatr*. 46, 10-4.

Kotloff, K. L., Winickoff, J. P., Ivanoff, B., Clemens, J. D., Swerdlow, D. L., Sansonetti, P. J., Adak, G. K., Levine, M. M., 1999. Global burden of Shigella infections: implications for vaccine development and implementation of control strategies. *Bull World Health Organ*. 77, 651-66.

Lin, W. S., Cheng, C. M., Van, K. T., 2010. A quantitative PCR assay for rapid detection of Shigella species in fresh produce. *J Food Prot*. 73, 221-33.

Lluque, A., Mosquito, S., Gomes, C., Riveros, M., Durand, D.,Tilley, D. H., Bernal, M., Prada, A., Ochoa, T. J., Ruiz, J., 2015. Virulence factors and mechanisms of antimicrobial resistance in Shigella strains from periurban areas of Lima (Peru). *Int J Med Microbiol*. 305, 480-90.

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., Abraham, J., Adair, T., Aggarwal, R., Ahn, S. Y., Alvarado, M., Anderson, H. R., Anderson, L. M., Andrews, K. G., Atkinson, C., Baddour, L. M., Barker-

Collo, S., Bartels, D. H., Bell, M. L., Benjamin, E. J., Bennett, D., Bhalla, K., Bikbov, B., Bin Abdulhak, A., Birbeck, G., Blyth, F., Bolliger, I., Boufous, S., Bucello, C., Burch, M., Burney, P., Carapetis, J., Chen, H., Chou, D., Chugh, S. S., Coffeng, L. E., Colan, S. D., Colquhoun, S., Colson, K. E., Condon, J., Connor, M. D., Cooper, L. T., Corriere, M., Cortinovis, M., de Vaccaro, K. C., Couser, W., Cowie, B. C., Criqui, M. H., Cross, M., Dabhadkar, K. C., Dahodwala, N., De Leo, D., Degenhardt, L., Delossantos, A., Denenberg, J., Des Jarlais, D. C., Dharmaratne, S. D., Dorsey, E. R., Driscoll, T., Duber, H., Ebel, B., Erwin, P. J., Espindola, P., Ezzati, M., Feigin, V., Flaxman, A. D., Forouzanfar, M. H., Fowkes, F. G., Franklin, R., Fransen, M., Freeman, M. K., Gabriel, S. E., Gakidou, E., Gaspari, F., Gillum, R. F., Gonzalez-Medina, D., Halasa, Y. A., Haring, D., Harrison, J. E., Havmoeller, R., Hay, R. J., Hoen, B., Hotez, P. J., Hoy, D., Jacobsen, K. H., James, S. L., Jasrasaria, R., Jayaraman, S., Johns, N., Karthikeyan, G., Kassebaum, N., Keren, A., Khoo, J. P., Knowlton, L. M., Kobusingye, O., Koranteng, A., Krishnamurthi, R., Lipnick, M., Lipshultz, S. E., Ohno, S. L., Mabweijano, J., MacIntyre, M. F., Mallinger, L., March, L., Marks, G. B., Marks, R., Matsumori, A., Matzopoulos, R., Mayosi, B. M., McAnulty, J. H., McDermott, M. M., McGrath, J., Mensah, G. A., Merriman, T. R., Michaud, C., Miller, M., Miller, T. R., Mock, C., Mocumbi, A. O., Mokdad, A. A., Moran, A., Mulholland, K., Nair, M. N., Naldi, L., Narayan, K. M., Nasseri, K., Norman, P., O'Donnell, M., Omer, S. B., Ortblad, K., Osborne, R., Ozgediz, D., Pahari, B., Pandian, J. D., Rivero, A. P., Padilla, R. P., Perez-Ruiz, F., Perico, N., Phillips, D., Pierce, K., Pope III, C. A., Porrini, E., Pourmalek, F., Raju, M., Ranganathan, D., Rehm, J. T., Rein, D. B., Remuzzi, G., Rivara, F. P., Roberts, T., De Leon, F. R.,

Rosenfeld, L. C., Rushton, L., Sacco, R. L., Salomon, J. A., Sampson, U., Sanman, E., Schwebel, D. C., Segui-Gomez, M., Shepard, D. S., Singh, D., Singleton, J., Sliwa, K., Smith, E., Steer, A., Taylor, J. A., Thomas, B., Tleyjeh, I. M., Towbin, J. A., Truelsen, T., Undurraga, E. A., Venketasubramanian, N., Vijayakumar, L., Vos, T., Wagner, G. R., Wang, M., Wang, W., Watt, K., Weinstock, M. A., Weintraub, R., Wilkinson, J. D., Woolf, A. D., Wulf, S., Yeh, P. H., Yip P., Zabetian, A., Zheng, Z. J., Lopez, A. D., Murray, C. J., AlMazroa, M. A., Memish, Z. A., 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 380, 2095-128.

Mandomando, I, Jaintilal, D, Pons, M.J., Vallès, X., Espasa, M., Mensa, L. Sigaúque, B., Sanz, S., Sacarlal, J., Macete, E., Abacassamo, F., Alonso, P.L., Ruiz, J. 2009. Antimicrobial susceptibility and mechanisms of resistance in *Shigella* and *Salmonella* isolates from children under five years of age with diarrhea in rural Mozambique. Antimicrob Agents Chemother. 53, 2450-4.

Mendeiros, P.H.Q.S., Lima, A.A.M, Guedes, M.M., Havt, A., Bona, M.D., Rey, L.C., Soares, A.M., Guerrant, R.L., Weigl, B.H., Lima, I.F.N. 2018. Molecular characterization of virulence and antimicrobial resistance profile of *Shigella* species isolated from children with moderate to severe diarrhea in northeastern Brazil. *Diagn Microbiol Infect Dis*. 90, 198-205.

Niyogi, S. K. 2005. Shigellosis. *J Microbiol*. 43, 133-43.

Qu, M., Zhang, X., Guirong, L., Huang, Y., Jia, L., Liang, W., Li, X., Wu, X., Li, J., Yan, H., Kan, B., Wang, Q. 2014. An eight-year study of *Shigella* species

in Beijing, China: serodiversity, virulence genes, and antimicrobial resistance. *J Infect Dev Ctries*. 8, 904-8.

Rosewell, A., Ropa, B., Posanai, E., Dutta, S. R., Mola, G., Zwi, A., MacIntyre, C. R., 2010. Shigella spp. antimicrobial drug resistance, Papua New Guinea, 2000-2009. *Emerg Infect Dis*. 16, 1797-9.

Schroeder, G. N., Hilbi, H., 2008. Molecular pathogenesis of Shigella spp.: Controlling host cell signaling, invasion, and death by type III secretion. *Clinical Microbiology Reviews*. 21, 134-156.

Soli, K. W., Maure, T., Kas, M. P., Bande, G., Bebes, S., Luang-Suarkia, D., Siba, P. M., Morita, A., Umezaki, M., Greenhill, A. R., Horwood, P. F., 2014. Detection of enteric viral and bacterial pathogens associated with paediatric diarrhoea in Goroka, Papua New Guinea. *Int J Infect Dis*. 27, 54-8.

Storch, G. A., Gunn, R. A., Martin, W. T., Pollard, R. A., Sinclair, S. P., 1980. Shigellosis in the Marshall Islands: epidemiologic aspects of an outbreak. *Am J Trop Med Hyg*. 29, 456-63.

Taneja, N., Mewara, A., 2016. Shigellosis: Epidemiology in India. *Indian J Med Res*. 143, 565-76.

Thong, K. L., Hoe, S. L., Puthucheary, S. D., Yasin, R., 2005. Detection of virulence genes in Malaysian Shigella species by multiplex PCR assay. *BMC Infect Dis*. 5, 8.

Watson, C., 2006. Death from multi-resistant shigelloses: a case study from Fiji. *Pac Health Dialog*. 13, 111-4.

Zhang, J., Qian, L., Wu, Y., Cai, X., Li, X., Cheng, X., Qu, D., 2013. Deletion of pic results in decreased virulence for a clinical isolate of Shigella flexneri 2a from China. *BMC Microbiol*. 13: 31.

Table 1. List of standard PCR primers used for the detection of *Shigella* virulence genes.

Gene target	Primers	Annealing temp. °C	Amplicon size (bp)	Reference
ipaH	ipaH_F1: GCTGGAAAAACTCAGTGCCT ipaH_R1: CCAGTCCGTAAATTCATTCT	56	424	Tornieporth et al., 1995
ial	ial_F: CTGGATGGTATGGTGAGG ial_R: GGAGGCCAACAATTATTTCC	58	320	Talukder et al., 2007
invE	invE_F: CGATAGATGGCGAGAAATTATATCCCG invE_R: CGATCAAGAATCCCTAACAGAAGAATCAC	57	766	Muller et al., 2007
ipaBCD	ipaBCD_F: GCTATAGCAGTGACATG ipaBCD_R: ACGAGTTCGAAGCACTC	55	500	Faruque et al., 2002
sen/ospD3	sen/ospD3_F: ATGTGCCTGCTATTATTTAT sen/ospD3_R: CATAATAATAAGCGGTCAGC	54	799	Talukder et al., 2007
set1A	set1A_F: TCACGCTACCATCAAAGA set1A_R: TATCCCCCTTTGGTGGTA	57	309	Talukder et al., 2007
set1B	set1B_F: GTGAACCTGCTGCCGATATC set1B_R: ATTAGTGGATAAAAATGACG	57	147	Talukder et al., 2007
virF	virF_F: TCAGGCAATGAAACTTTGAC virF_R: TGGGCTTGATATTCCGATAAGTC	58	618	Gomez-Duarte et al., 2009

12 **Table 2.** Virulence profiles based on presence/absence of virulence genes in *Shigella* by species

Species	*ipaH*	*sen/osp D3*	*set1A*	*set1B*	*invE*	*ial*	*ipaBCD*	*virF*	Year of Isolation	No. Isolates	Serotype	Biotype	Origin
S. flexneri	+	+	-	-	+	+	+	+	1985	1	3		PNG
									1994	1	2a		PNG
									2002	1	6		PNG
									2006	1	3a		PNG
									2006	1	2a		PNG
									2007	1	3a		PNG
									2007	1	1b		Fiji
									2010	4	nt		PNG
									2011	1	nt		PNG
									2012	1	X		Samoa
S. flexneri	+	-	-	-	-	-	-	-	1985	3	1		PNG
									1985	1	3		PNG
									1992	1	nt		PNG
									2000	1	2a		PNG
									2004	1	1b		PNG
									2010	3	nt		PNG
S. flexneri	+	-	+	+	-	-	-	-	2009	1	2a		PNG
S. flexneri	+	+	+	+	+	+	+	+	2009	1	2a		Torres Strait
									2010	1	2a		Torres Strait

									2010	20	nt		PNG
									2011	2	2a		PNG
													Torres
									2013	1	2a		Strait
									2013	3	2a		PNG
									2014	1	X		PNG
									1999	1		a	PNG
													Solomon
									2007	1		a	Islands
									2008	1		g	PNG
									2011	2		g	PNG
S. sonnei	+	-	-	-	-	-	-	-	2012	3		a	Fiji
									2013	1		a	Samoa
									2013	1		a	Vanuatu
									2014	3		g	PNG
									2015	1		g	PNG
									2015	1		a	Vanuatu
S. sonnei	+	+	-	-	+	+	+	+	2010	1		f	PNG
S. dysenteriae	+	-	-	-	-	-	-	-	1985	1	nt		PNG
									2010	2	nt		PNG

Legend: nt not tested; PNG Papua New Guinea

Table 3. Proportion of *Shigella* isolates resistant to selected antimicrobials.

Number of *Shigella* species (%) with – antimicrobial resistance				
Antimicrobial	*S. flexneri* (n=53)	*S. sonnei* (n=16)	*S. dysenteriae* (n=3)	Total (%) (n=72)
AMP	41 (77)	9 (56)	1 (33)	51 (71)
CHL	32 (60)	0	0	32 (44)
CIP	0	0	0	0
CRO	0	0	0	0
NAL	0	1 (6)	0	1 (1)
TET	39 (74)	3 (19)	1 (33)	43 (60)
SXT	26 (49)	12 (75)	1 (33)	39 (54)

Legend: Ampicillin (AMP), Chloramphenicol (CHL), Ciprofloxacin (CIP), Nalidixic acid (NAL), Tetracycline (TET), trimethoprim-sulfamethoxazole (SXT).

Table 4. Antibiotic resistance profiles of *Shigella* isolates.

	S. flexneri	*S. sonnei*	*S. dysenteriae*	Total (%)
AMP/CHL/TET/ SXT	17 (32)	0	0	17 (24)
AMP/CHL/TET	12 (23)	0	0	12 (17)
AMP/SXT	1 (2)	7 (44)	1 (33)	9 (13)
AMP/TET/SXT	4 (8)	2 (13)	0	6 (8)
AMP/TET	3 (6)	0	0	3 (4)
CHL/TET/SXT	2 (4)	0	0	2 (3)
AMP/CHL/SXT	1 (2)	0	0	1 (1)
NAL/TET/SXT	0	1 (6)	0	1 (1)
AMP	3 (6)	0	0	3 (4)
TET	1 (2)	0	1 (33)	2 (3)
SXT	1 (2)	2 (13)	0	3 (4)
Susceptible	8 (15)	4 (25)	1 (33)	13 (18)
Total	53	16	3	72

Figure 1. Proportion of isolates (%) isolated before and after 2010 that are susceptible or resistant to selected antibiotics. The post-2010 group includes isolates from 2010.