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Detection of enteric viral and bacterial pathogens associated with paediatric diarrhoea in Goroka, Papua New Guinea

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SUMMARY

Objectives: The aim of this study was to investigate the viral and bacterial causes of acute watery diarrhoea in hospitalized children in Papua New Guinea.
Methods: A retrospective analysis was conducted on stool samples collected from 199 children (age <5 years) admitted to the paediatric ward of Goroka General Hospital from August 2009 through November 2010. A large range of viral and bacterial enteric pathogens were targeted using real-time PCR/RT-PCR assays.
Results: Young children were much more likely to be admitted with acute gastroenteritis, with 62.8% of patients aged <1 year and 88.4% aged <2 years. An enteric pathogen was detected in 69.8% (n = 138) of patients. The most commonly detected pathogens were Shigella *spp* (26.6%), rotavirus (25.6%), adenovirus types 40/41 (11.6%), enterotoxigenic *Escherichia coli* (11.1%), enteropathogenic *E. coli* (8.5%), norovirus G2 (6.0%), and *Campylobacter spp* (4.0%). Norovirus G1, sapovirus, and *Salmonella spp* were also detected, but below our statistical limit of detection. *Vibrio cholerae* and astrovirus were not detected in any patients. Mixed infections were detected in 22.1% of patients, with Shigella and rotavirus most commonly detected in co-infections with other pathogens.
Conclusions: This study demonstrates that Shigella and rotavirus are the major pathogens associated with acute paediatric gastroenteritis in this setting.

1. Introduction

Diarrhoeal illnesses are a major cause of morbidity and mortality in children worldwide. An estimated 1.8 million children die each year from largely preventable enteric illnesses, with the large majority of these mortalities occurring in developing countries.[1] Rotavirus is the most important cause of acute gastroenteritis in children worldwide, with recent estimates suggesting that more than 1.4 million hospital admissions and 500 000 deaths can be attributed to rotavirus infections annually.[2] However, many viral, bacterial, and protozoan pathogens are associated with diarrhoeal illness in children, and this broad range of pathogens involved in childhood diarrhoea contributes to challenges in overcoming the high burden of disease.

In Papua New Guinea, enteric illnesses are one of the major causes of outpatient visits, hospitalizations, and mortalities, particularly in young children.[3] However, poor diagnostic capacity has greatly limited knowledge pertaining to the aetiology and epidemiology of enteric illnesses in this setting.[4] Past studies have established rotavirus as an important cause of acute gastroenteritis in children in this setting,[5,6] and outbreaks of cholera[7] and shigellosis[8] have also affected many communities. However, little effort has been made in recent years to fully comprehend the aetiology of childhood enteric illness in Papua New Guinea by way of detection of multiple aetiologies, with the most recent comprehensive study conducted on samples that were collected approximately 25 years ago.[5] In this study, we investigated the

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aetiology of acute watery diarrhoea in hospitalized children in Goroka, Papua New Guinea.

2. Materials and methods

2.1. Sample collection

Stool samples were collected from children <5 years of age hospitalized with acute gastroenteritis at Goroka General Hospital, Papua New Guinea from August 2009 through November 2010. Goroka is the provincial capital of the Eastern Highlands Province of Papua New Guinea, and Goroka General Hospital is the major referral hospital for the region and has a catchment population of approximately 450 000 people. Children were considered eligible for inclusion in the study if their parent/guardian reported acute watery diarrhoea (>3 loose bowel movements in the last 24 h) and no blood or mucous was observed in the faeces. Dysentery samples were excluded from analysis because sample collection was designed for acute watery diarrhoea surveillance as part of the Western Pacific Region Rotavirus Surveillance Network (World Health Organization). All samples were collected within 48 h of hospitalization and transported to the laboratory at 4 °C and then aliquoted and stored at −80 °C.

2.2. Detection of enteric pathogens

Nucleic acids were extracted from the stool samples using the Qiagen DNA Stool Mini Kit (Qiagen, Germany) in accordance with the manufacturer's instructions. Eluates were stored at −80 °C until required for testing.

Eluates from the stool samples were tested for a range of viral and bacterial enteric pathogens using real-time PCR/RT-PCR assays (Table 1).[27–33] RNA viruses (rotavirus, norovirus G1 and G2, sapovirus, and astrovirus) were detected in individual reactions using the QuantiTect Multiplex Real-Time RT-PCR Mastermix (Qiagen, Germany). DNA viruses (adenovirus types 40/41) and bacteria (Shigella *spp*, *Salmonella spp*, *Vibrio cholerae*, *Campylobacter spp*, enteropathogenic *Escherichia coli* (EPEC) and enterotoxigenic *E. coli* (ETEC)) were detected in individual reactions using the QuantiTect Multiplex Real-Time PCR Mastermix (Qiagen, Germany). All reaction conditions were those given in the Mastermix manufacturer's instructions. Negative and positive controls were included in each run to ensure the validity of results.

2.3. Sample size and statistics

We calculated sample size based on historical data from this setting, where the most important pathogens were detected in at least 4% of cases.[5] To enable detection of pathogens with a prevalence of 8% to a precision of ±4% at a 95% confidence level, we required a sample size of 177. Pathogen prevalence was calculated in Microsoft Excel and expressed as a percentage of positive samples in relation to the total number of samples tested.

3. Results

Stool samples were collected from 199 children <5 years of age (109 male and 90 female) admitted with acute gastroenteritis to Goroka General Hospital. The age range of the children was 1–60 months, with a median age of 10 months. There was no significant difference in the detection of any enteric pathogens between males and females (Fisher's exact test, data not shown). Analysis of the age distribution of infections between children aged <1 year ($n = 125$) and children aged 1–5 years ($n = 74$) showed no significant difference in the detection rate of pathogens between the two age groups (Fisher's exact test, data not shown). Young children were much more likely to be admitted to hospital with acute gastroenteritis, with 62.8% of patients aged <1 year and 88.4% aged <2 years.

An enteric pathogen was detected in 69.8% ($n = 138$) of stool samples collected from children hospitalized with acute gastroenteritis. Table 2 shows the distribution of pathogens found in the stool samples. Shigella *spp* (26.6%, $n = 53$), rotavirus (25.6%, $n = 51$), and adenovirus 40/41 (11.6%, $n = 23$) were the enteric pathogens most commonly detected. EPEC strains were detected in 8.5% ($n = 17$) of patients. The majority ($n = 13$) of the EPEC strains detected were classified as atypical, based on the absence of the EPEC adherence factor (EAF) plasmid. ETEC strains were detected in 11.1% ($n = 22$) of the samples, with the ETEC toxin genes ST and LT detected in 7% ($n = 14$) and 6% ($n = 12$) of samples, respectively. Both toxin genes were detected in four samples; however, without isolation of *E. coli* strains we cannot ascertain whether multiple strains were present in individual samples or single strains contained both toxin genes. *Campylobacter spp* (4.0%, $n = 8$) was the only other pathogen detected within the limits of detection for this study; other enteric pathogens such as norovirus G1, norovirus G2, sapovirus, and *Salmonella spp* occurred sporadically (in <10% of patients) but below our statistical limit of detection. Astrovirus and *V. cholerae* were not detected in any patients. Mixed infections were detected in 22.1% ($n = 44$) of patients, with Shigella (13.1%, $n = 26$) and rotavirus (11.1%, $n = 22$) the pathogens most commonly detected in mixed infections. Other co-infections were also detected at a high frequency (Tables 2 and 3).

4. Discussion

We have clearly demonstrated the importance of Shigella and rotavirus in childhood enteric illness in the highlands of Papua New Guinea. These results support our recently published findings in the same setting investigating the prevalence and epidemiology of rotavirus in children,[6] and the high prevalence of Shigella detected by culture in adults and children seeking outpatient medical assistance.[9] The findings are also consistent with global trends, with a recent multicentre case-controlled study finding Shigella, rotavirus, and ETEC to be commonly associated with moderate to severe childhood diarrhoea.[10]

We did not speciate Shigella in this study; however, our recent study in adults and children showed Shigella *flexneri* to be the most commonly isolated species.[9] Other studies in this setting have shown that antimicrobial resistance in Shigella strains is a major concern, with multidrug resistance detected in the majority of samples.[8,9] The World Health Organization now recommends ciprofloxacin as the drug of choice for all patients with dysentery;[11] to date resistance to ciprofloxacin has not been reported in Papua New Guinea.

In this study we did not type rotavirus, however the globally circulating strains G1P[8], G3P[8], and G2P[4] were recently demonstrated to be the most prevalent in this setting.[6] Routine rotavirus vaccination has not been initiated in Papua New Guinea, so pre-existing immunity due to vaccination is unlikely to have influenced the incidence of rotaviral disease.

Enteric parasites were not targeted, but it is likely that pathogens such as *Cryptosporidium spp* and *Giardia spp* play an important role in gastrointestinal illness in children in this setting. A study by Phuanukoonnon and colleagues[12] found Giardia to be common in pregnant women in the highlands of Papua New Guinea; so transmission to children would seem likely. In other low-income settings *Cryptosporidium spp* are important pathogens, as demonstrated in the recent case-controlled multicentre study.[10] Further parasitological investigations are warranted in this setting,

Table 1
Primers used for the real-time PCR/RT-PCR detection of enteric pathogens

Pathogen	Primer/probe	Sequence	Reference
Rotavirus	ROTF1	ACCATCTTCACGTAACCCTC	27
	ROTF2	ACCATCTACACATGACCCTC	
	ROTR	CACATAACGCCCCTATAGCC	
	ROTP	FAM-ATGAGCACAATAGTTAAAAGCTAACACTGTCAA-BHQ1	
Norovirus G1	NOG1F	CGYTGGATGCGNTTYCATGA	27
	NOG1R	CCTTAGACGCCATCATCATTTAC	
	NORG1P	VIC-TYGCGRTCTCCTGTCCA-MGBNFQ	
Norovirus G2	NOG2F	CARGARBCNATGTTYAGRTGGATGAG	27
	NOG2R	TCGACGCCATCTTCATTCACA	
	NOG2P	VIC-AGATYGCGATCSCCCTC-MGBNFQ	
Astrovirus	ASTF	TCTYATAGACCGYATTATTGG	27
	ASTR	TCAAATTCTACATCATCACCAA	
	ASTP	Cy5-CCCCADCCATCATCATCTTCATCA-BHQ3	
Sapovirus	SAPF	CAGGCTCTCGCCACCTAC	27
	SAPR	CCCTCCATYTCAAACACTAWTTT	
	SAPP	FAM-TGGTTCATAGGTGGTRC-MGBFNQ	
Adenovirus (40/41)	AdF40–41	TTCCAGCATAATAACTCWGGCTTTG	28
	AdR40–41	AATTTTTTCTGWGTCAGGCTTGG	
	advMGB	FAM-CCWTACCCCCTTATTGG-MGBNFQ	
Shigella *spp*	ShigF	ACCATGCTCGCAGAGAAACT	29
	ShigR	TACGCTTCAGTACAGCATGC	
	ShigP	HEX-TGGCGTGTCGGGAGTGACAGC-BHQ1	
Salmonella spp	SAompF	CCTGGCAGCGGTGATCC	30
	SAompR	AAATTTCTGCTGCGTTTGCG	
	SAompP	FAM-TGCCCTGCTGGCTGCTGCA-MGBNFQ	
Campylobacter spp	CampF	CTGCTTAACACAAGTTGAGTAGG	31
	CampR	TTCCTTAGGTACCGTCAGAA	
	CampP	FAM-TGTCATCCTCCACGCGGCGTTGCTGC-BHQ1	
Vibrio cholerae	VCctxF	TTTGTTAGGCACGATGATGGAT	32
	VCctxR	ACCAGACAATATAGTTTGACCCACTAAG	
	VCctxP	FAM-TGTTTCCACCTCAATTAGTTTGAGAAGTGCCC-BHQ1	
EPEC (*eae* gene)	EAE-F	ACTGGACTTCTTATTRCCGTTCTATG	33
	EAE-R1	CTAAGCGGGTATTGTTACCAGA	
	EAE-R2	CCTAAACGGGTATTATCACCAGA	
	EAE-P	TxRd-AATCCTGATCAATGAAGACGTTATAGCCCA-BHQ2	
EPEC (EAF plasmid)	EPS-F	GTTCTTGGCGAACAGGCTTGTC	33
	EPS-R	TTAAGCCAGCTACCATCCACCC	
	EPS-P	Cy5-AGTACTGACGTGCAGGTCGCCTGTTCG-BHQ3	
ETEC (ST)	ST-F	CTGGTTTTGATTCAAATGTTCGTG	33
	ST-R	TCCTGAGGGAAAGGTGAAAAAGAC	
	ST-P	TxRd-TTGATTTCTTCATATTACCTCCGGACATGGCA-BHQ2	
ETEC (LT)	LT-F	AGCGGCGCAACATTTCAG	33
	LT-R	TTGGTCTCGGTCAGATATGTGATTC	
	LT-P	FAM-TCGAAGTCCCGGGCAGTCAACATATAGA-BHQ1	

EPEC, enteropathogenic *Escherichia coli*; EAF, EPEC adherence factor; ETEC, enterotoxigenic *Escherichia coli*.

particularly given that parasitic gastrointestinal infections may have impacts beyond enteric illness (e.g. childhood development).

Although this was not a case–control study, our investigation provides important data on the presence of enteric pathogens in children presenting with diarrhoeal illness. A previous case–control study by Howard et al.[5] detected rotavirus in 23% (odds ratio 18.2) and Shigella in 13% (odds ratio 9.6) of children hospitalized with diarrhoea. On this basis, and the established knowledge that these are globally important enteric pathogens, we are confident that these pathogens are the major contributors to childhood diarrhoeal illness in this setting. The detection of Shigella at a higher rate than rotavirus and all other pathogens in the present study is noteworthy, particularly as children with blood or mucous in their stools were omitted from the study. Acute watery diarrhoea is commonly reported as the initial phase of shigellosis, preceding dysentery, and is often the only symptom during mild infections.[13] In Papua New Guinea, Shigella seems to be very prevalent in the community and dysentery outbreaks are frequently reported. The importance of Shigella in Papua New Guinea is exemplified by a recent outbreak of Shigella in an internally displaced population, which spread to the surrounding community and resulted in approximately 1200 cases of shigellosis and 5 deaths.[34]

Mixed infections were detected at a high frequency in this study (22.1%), which is comparable to the level of co-infections detected in a diarrhoeal aetiology study conducted in Tanzania (20.7%)[14] and consistent with the high level of co-infections detected in other developing settings such as Jordan (15.5%),[15] Libya (13.8%),[16] Vietnam (13.5%),[17] and Brazil (11.0%).[18] In comparison, co-infections are typically reported at lower frequency in developed settings, e.g. Italy (9.8%),[19] Denmark (1.9%),[20] and France (1.1%).[21] These findings may be associated with increased levels of enteric pathogens in the environment due to poor levels of sanitation and hygiene in developing settings.

A recent study estimated the incidence of paediatric diarrhoea in 140 countries.[1] The study indicated that Papua New Guinea had the highest incidence of paediatric diarrhoea in the Western Pacific Region for all age groups (0–5, 6–11, 12–23, and 24–59 months). These figures may be a reflection of the poor access to safe water sources and improved sanitation (approximately 40% and 45% coverage, respectively) throughout the country.[22] Interventions to target all enteric illness are urgently required in Papua New Guinea and other developing settings, and improved sanitation and hygiene would greatly contribute to decreasing the burden of these illnesses.[23,24] Further interventions such as educational campaigns to improve hygiene, hand-washing with soap, and exclusive breastfeeding in young infants should also be considered, as they have been shown to significantly reduce enteric illnesses in children.[25,26] Moreover, further study and targeting of specific

Table 2
The frequency of pathogen detection in children (aged <5 years) hospitalized with acute gastroenteritis

Characteristics	Total (n = 199)
Tested positive for a pathogen	138 (69.8%)
Multiple pathogens detected	44 (22.1%)
Shigella *spp*	53 (26.6%)
Rotavirus	51 (25.6%)
Adenovirus 40/41	23 (11.6%)
Enterotoxigenic *E. coli* (ETEC)	22 (11.1%)
Enteropathogenic *E. coli* (EPEC)	17 (8.5%)
Norovirus G2	12 (6.0%)
Campylobacter spp	8 (4.0%)
Norovirus G1	7 (3.5%)[b]
Sapovirus	4 (2.0%)[b]
Salmonella spp	2 (1.0%)[b]
Vibrio cholerae	0 (0%)
Astrovirus	0 (0%)
Co-infections[a]	
Shigella *sp* and rotavirus	10 (5.0%)
Shigella *sp* and ETEC	8 (4.0%)
Rotavirus and adenovirus 40/41	5 (2.5%)
Rotavirus and norovirus G1	3 (1.5%)
Norovirus G2 and EPEC	3 (1.5%)
Shigella *sp* and EPEC	3 (1.5%)
Shigella *sp* and adenovirus 40/41	3 (1.5%)
Shigella *sp* and *Campylobacter spp*	2 (1.0%)
Shigella *sp* and norovirus G2	2 (1.0%)
Rotavirus and *Campylobacter spp*	2 (1.0%)
Rotavirus and ETEC	2 (1.0%)

[a] Only co-infections detected in multiple patients are listed; pathogen associations also include instances where more than two pathogens were detected in a sample.
[b] These pathogens were detected at levels below our statistical limit of detection.

Table 3
Total number of co-infections for specific pathogens

Pathogen	Number of co-infections (%[a])
Shigella *spp*	26 (49.1)
Rotavirus	22 (43.1)
Enterotoxigenic *E. coli*	11 (50.0)
Adenovirus 40/41	10 (43.5)
Enteropathogenic *E. coli*	7 (41.2)
Norovirus G2	6 (50.0)
Norovirus G1	4 (57.1)
Campylobacter spp	4 (50.0)
Salmonella spp	1 (50.0)
Sapovirus	1 (25.0)

[a] The proportion of occasions that a pathogen was associated with a co-infection.

pathogens such as Shigella and rotavirus, and determining the interactions between major and lesser enteric pathogens, may also be warranted in the future.

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Ethical approval: This study was approved by the Papua New Guinea Institute of Medical Research Institutional Review Board and the Papua New Guinea Medical Research Advisory Council. All samples were collected following informed consent from parents or guardians.

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References

1. Fischer Walker CL, Perin J, Aryee MJ, Bos Chi-Pinto C, Black RE. Diarrhea incidence in low- and middle-income countries in 1990 and 2010: a systematic review. *BMC Public Health* 2012;**12**:220–6.
2. Parashar UD, Gibson CJ, Bresee JS, Glass RI. Rotavirus and severe childhood diarrhoea. *Emerg Infect Dis* 2006;**12**:304–6.
3. National Health Plan 2001–2010: Health Vision 2010. *Policy directions and priorities*, 1. Port Moresby: Papua New Guinea Ministry of Health; 2000.
4. Greenhill AR, Rosewell A, Kas M, Manning M, Siba PM, Horwood PF. Improved laboratory capacity is required to better respond to future cholera outbreaks in Papua New Guinea. *Western Pac Surveill Response J* 2012;**3**:30–2.
5. Howard P, Alexander ND, Atkinson A, Clegg AO, Gerega G, Javati A, et al. Bacterial, viral and parasitic aetiology of paediatric diarrhoea in the highlands of Papua New Guinea. *J Trop Pediatr* 2000;**46**:10–4.
6. Horwood PF, Luang-Suarkia D, Bebes S, Boniface K, Datta SS, Siba PM, et al. Surveillance and molecular characterization of group A rotaviruses in Goroka, Papua New Guinea. *Am J Trop Med Hyg* 2012;**87**:1145–8.
7. Horwood PF, Collins D, Jonduo MH, Rosewell A, Dutta SR, Dagina R, et al. Clonal origins of *Vibrio cholerae* O1 El Tor strains, Papua New Guinea, 2009-2011. *Emerg Infect Dis* 2011;**17**:2063–5.
8. Rosewell A, Ropa B, Posanai E, Dutta SR, Mola G, Zwi A, et al. *Shigella spp*. antimicrobial drug resistance, Papua New Guinea, 2000-2009. *Emerg Infect Dis* 2010;**16**:1797–9.
9. Greenhill AR, Guwada C, Siba V, Michael A, Yoannes M, Wawarie Y, et al. Antibiotic resistant Shigella is a major cause of diarrhoea in the Highlands of Papua New Guinea. *J Infect Dev Ctries* 2014. in press.
10. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case–control study. *Lancet* 2013;**382**:209–22.
11. World Health Organization. Guidelines for the control of shigellosis, including epidemics due to *Shigella dysenteriae* type 1. Geneva: WHO; 2005. Available at: http://www.who.int/topics/cholera/publications/shigellosis/en/index.html (accessed January 28, 2014).
12. Phuanukoonnon S, Michael A, Kirarock WS, Pomat W, van den Biggelaar AH. Intestinal infections and anaemia among pregnant women in the Highlands of Papua New Guinea. *PNG Med J* 2013. in press.
13. Niyogi SK. *Shigellosis J Microbiol* 2005;**43**:133–43.
14. Moyo SJ, Gro N, Matee MI, Kitundu J, Myrmel H, Mylvaganam H, et al. Age specific aetiological agents of diarrhoea in hospitalized children aged less than five years in Dar es Salaam, Tanzania. *BMC Pediatr* 2011;**11**:19.
15. Youssef M, Shurman A, Bougnoux M, Rawashdeh M, Bretagne S, Strockbine N. Bacterial, viral and parasitic enteric pathogens associated with acute diarrhea in hospitalized children from northern Jordan. *FEMS Immunol Med Microbiol* 2000;**28**:257–63.
16. Rahouma A, Klena JD, Krema Z, Abobker AA, Treesh K, Franka E, et al. Enteric pathogens associated with childhood diarrhea in Tripoli-Libya. *Am J Trop Med Hyg* 2011;**84**:886–91.
17. Vu Nguyen T, Le Van P, Le Huy C, Nguyen Gia K, Weintraub A. Etiology and epidemiology of diarrhea in children in Hanoi, Vietnam. *Int J Infect Dis* 2006;**10**:298–308.
18. Moreno AC, Filho AF, Gomes Tdo A, Ramos ST, Montemor LP, Tavares VC, et al. Etiology of childhood diarrhea in the northeast of Brazil: significant emergent diarrheal pathogens. *Diagn Microbiol Infect Dis* 2010;**66**:50–7.
19. Colomba C, De Grazia S, Giammanco GM, Saporito L, Scarlata F, Titone L, et al. Viral gastroenteritis in children hospitalised in Sicily, Italy. *Eur J Clin Microbiol Infect Dis* 2006;**25**:570–5.
20. Olesen B, Neimann J, Böttiger B, Ethelberg S, Schiellerup P, Jensen C, et al. Etiology of diarrhea in young children in Denmark: a case–control study. *J Clin Microbiol* 2005;**43**:3636–41.
21. Lorrot M, Bon F, El Hajje MJ, Aho S, Wolfer M, Giraudon H, et al. Epidemiology and clinical features of gastroenteritis in hospitalised children: prospective survey during a 2-year period in a Parisian hospital, France. *Eur J Clin Microbiol Infect Dis* 2011;**30**:361–8.
22. World Health Organization, United Nations Children's Fund Joint Monitoring Programme for Water Supply and Sanitation. Estimates for the use of improved sanitation facilities, Papua New Guinea. WHO/UNICEF; 2010. Available at: http://www.unicef.org/infobycountrypapuang_statistics.html (accessed November 14, 2011).
23. Horwood PF, Greenhill AR. Cholera in Papua New Guinea and the importance of safe water sources and sanitation. *Western Pac Surveill Response J* 2012;**3**:3–5.
24. Horwood PF, Barrington DJ, Greenhill AR. More resources need to be committed to the provision of safe water sources and sanitation in Papua New Guinea. *PNG Med J* 2014. in press.
25. Cairncross S, Hunt C, Boisson S, Bostoen K, Curtis V, Fung IC, et al. Water, sanitation and hygiene for the prevention of diarrhoea. *Int J Epidemiol* 2010;**39**(Suppl 1):i193–205.

26. Duijts L, Ramadhani MK, Moll HA. Breastfeeding protects against infectious diseases during infancy in industrialized countries. A systematic review. *Matern Child Nutr* 2009;**5**:199–210.
27. Van Maarseveen NM, Wessels E, de Brouwer CS, Vossen AC, Claas EC. Diagnosis of viral gastroenteritis by simultaneous detection of adenovirus group F, astrovirus, rotavirus group A, norovirus genogroups I and II, and sapovirus in two internally controlled multiplex real-time PCR assays. *J Clin Virol* 2010;**49**:205–10.
28. Logan C, O'Leary JJ, O'Sullivan N. Real-time reverse transcription-PCR for detection of rotavirus and adenovirus as causative agents of acute viral gastroenteritis in children. *J Clin Microbiol* 2006;**44**:3189–95.
29. Lin WS, Cheng CM, Van KT. A quantitative PCR assay for rapid detection of Shigella species in fresh produce. *J Food Prot* 2010;**73**:221–33.
30. Tatavarthy A, Cannons A, Real-time PCR. detection of *Salmonella* species using a novel target: the outer membrane porin F gene (*omp*F). *Lett Appl Microbiol* 2010;**50**:645–52.
31. Josefsen MH, Jacobsen NR, Hoorfar J. Enrichment followed by quantitative PCR both for rapid detection and as a tool for quantitative risk assessment of food-borne thermotolerant campylobacters. *Appl Environ Microbiol* 2004;**70**:3588–92.
32. Blackstone GM, Norstrom JL, Bowen MD, Meyer RF, Imbro P, DePaola A. Use of a real time PCR assay for detection of the *ctx*A gene of *Vibrio cholerae* in an environmental survey of Mobile Bay. *J Microbiol Methods* 2007;**68**:254–9.
33. Hardegen C, Messler S, Henrich B, Pfeffer K, Würthner J, MacKenzie CR. A set of novel multiplex Taqman real-time PCRs for the detection of diarrhoeagenic *Escherichia coli* and its use in determining the prevalence of EPEC and EAEC in a university hospital. *Ann Clin Microbiol Antimicrob* 2010;**9**:5. http://dx.doi.org/10.1186/1476-0711-9-5.
34. Benny E, Mesere K, Pavlin BI, Yakam L, Ford R, Yoannes M, et al. A large outbreak of shigellosis commencing in an internally displaced population, Papua New Guinea, 2013. *Western Pac Surveill Response J*. In Press.