Analysis of *Vibrio cholerae* isolates from the Northern Cape province of South Africa

Vibrio cholerae is a natural inhabitant of the aquatic environment and can be transmitted to humans through drinking water. South Africa (SA) was recently affected by an epidemic of *V. cholerae* O1, which occurred from 1997 to 2005 and peaked in 2001 (Keddy *et al.*, 2007). This epidemic affected most provinces in the country, but was not described in the Free State province or the Northern Cape province. Vigilance for the disease remains high in the medical community and cases of *V. cholerae* infection should be investigated because of the public health importance of epidemic cholera. During the SA epidemic in 2000–2002, we identified a number of non-O1, non-O139 *V. cholerae* strains that were negative for cholera toxin, but were co-circulating in the environment (K. H. Keddy, unpublished results). Non-O1, non-O139 *V. cholerae* may be associated with mild diarrhoea and localized, contained outbreaks. Although these strains have low epidemic potential, they may be encapsulated and more frequently cause invasive disease. Their presence in the environment also suggests that environmental conditions are optimal for supporting the growth of epidemic *V. cholerae* O1, which is known to exist in groundwater sources in a viable but non-culturable resting state (Kaper *et al.*, 1995). In the present study, we report on the investigation of non-O1, non-O139 *V. cholerae* strains isolated in the Northern Cape province of SA during March to April 2007. These strains included one invasive human isolate and ten water isolates.

On 22 March 2007, *V. cholerae* was isolated from the blood culture of a 25-year-old female admitted to Kimberley Hospital, located in the Northern Cape province of SA. *Escherichia coli* was cultured from the blood simultaneously. The patient was severely immunosuppressed and presented with abdominal sepsis. She died shortly after hospital admission due to cardiac

arrest ascribed to septic shock and multiple organ failure. No further cases of *V. cholerae* isolation from humans were reported from this area. However, this single human isolation of *V. cholerae* was closely followed (26 March–8 April) by the isolation of *V. cholerae* strains from ten water samples taken at various sections of the nearby Vaal and Harts Rivers (Table 1). All 11 strains were sent to the Enteric Diseases Reference Unit for confirmation of *V. cholerae* diagnosis and for genotypic analysis. Bacterial strains were confirmed as *V. cholerae* using standard microbiological identification techniques. Serogrouping was attempted on all strains using the slide agglutination method with polyvalent antisera and mono-specific Inaba and Ogawa antisera, according to the manufacturer's instructions (Murex Biotech). Detection of the cholera enterotoxin gene (*ctxA*) was performed by PCR using a previously described method (Keasler & Hall, 1993). For this PCR, we used primers as described in the above method and employed 28 cycles of 95 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min. Susceptibility testing to antimicrobial agents (ampicillin, augmentin, trimethoprim, sulfamethoxazole, chloramphenicol, nalidixic acid, ciprofloxacin, tetracycline, kanamycin, streptomycin, imipenem, ceftriaxone, erythromycin and ceftazidime) was determined by the Etest (AB BIODISK), according to the manufacturer's instructions. PFGE analysis of strains was performed using a PulseNet standardized protocol (Cooper *et al.*, 2006) incorporating separate analysis with *Sfi*I and *Not*I restriction enzymes. The CHEF-DR III system (Bio-Rad Laboratories) was used for PFGE analysis, with an electrophoresis gradient of 6 V cm⁻¹ and a two-block run programmed as follows. Block 1 included an initial switch time (IST) of 2 s to a final switch time (FST) of 10 s, over a run time of 13 h; while block 2 included an IST of 20 s to a FST of 25 s,

over a run time of 7 h. Repetitive element sequence-based PCR (REP-PCR) analysis of strains was performed using previously described primers ERIC1R (ATGTAAGCTCCTGGGGATTTCAC) and ERIC2 (AAGTAAGTGACTGGGGTGAGCG) in a PCR employing four cycles of 95 °C for 5 min, 40 °C for 5 min and 72 °C for 5 min; followed by 30 cycles of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min (Versalovic *et al.*, 1991). REP-PCR profiles were generated following electrophoretic separation of PCR products on a 1.5% agarose gel. PFGE and REP-PCR profiles of strains were analysed and compared using the GelCompar II software, version 4.6 (Applied Maths). Dendrograms of the profiles were created using the unweighted pair group method with arithmetic averages, with analysis of banding patterns incorporating the Dice coefficient. Strains sharing ≥90% similarity on a dendrogram were defined as the same clone.

All *V. cholerae* strains autoagglutinated in saline, therefore serogroup status could not be determined. Autoagglutination was probably due to the presence of bacterial capsule. All strains were PCR-negative for the cholera enterotoxin gene (*ctxA*), which confirmed that this was not epidemic cholera and strongly suggested that these were *V. cholerae* non-O1, non-O139 serotypes. Human infections with *V. cholerae* non-O1, non-O139 serotypes are rare and sporadic cases are generally reported. Previous reports of *V. cholerae* non-O1, non-O139 septicaemia have included patients in Austria (Halabi *et al.*, 1997), Slovenia (Strumbelj *et al.*, 2005) and Poland (Stypulkowska-Misiurewicz *et al.*, 2006). Our present report describes another such sporadic case of septicaemia caused by non-O1, non-O139 *V. cholerae*. Our isolation of *V. cholerae* from a human was closely followed by the isolation of *V. cholerae* strains from ten water samples taken at

Table 1. Details of *V. cholerae* strains

NICD reference no.	Specimen collection date and time	Specimen source	Antimicrobial MIC ($\mu\text{g ml}^{-1}$)*													
			AMP	AUG	TRI	SUL	CHL	NAL	CIP	TET	KAN	STR	IMI	CRO	ERY	CAZ
TCD 190511	22/3/2007	Human (blood culture)	3	4	0.38	64	0.75	0.38	0.003	1	4	12	0.75	0.016	1	0.19
TCD 191499	26/3/2007, 13h15	Water (Vaalharts Weir)	3	6	0.38	16	1	0.25	0.002	1	4	6	2	0.016	1.5	0.125
TCD 191717	26/3/2007, 13h45	Water (Vaalharts Weir)	4	8	0.5	128	2	0.75	0.002	1	6	8	2	0.016	1.5	0.125
TCD 191718	27/3/2007, 14h35	Water (Schmidtsdrift)	>128	6	0.75	>1024	0.75	1.5	0.003	1.5	8	12	1	0.016	2	0.125
TCD 191723	27/3/2007, 11h35	Water (Barkley West)	1	2	0.38	64	0.75	0.19	0.002	1.5	4	12	0.5	0.016	1	0.094
TCD 191908	29/3/2007	Water (Spitskop Dam)	>256	6	0.5	>1024	0.38	1.5	0.002	1.5	4	12	0.75	0.016	1	0.125
TCD 192117	29/3/2007	Water (Spitskop Dam)	>256	6	0.5	>1024	1	1.5	0.002	1.5	6	12	1	0.016	1	0.19
TCD 192725	2/4/2007	Water (Schmidtsdrift)	0.75	2	0.25	1024	0.5	0.75	0.002	0.75	4	8	0.5	0.016	1.5	0.094
TCD 192727	4/4/2007, 12h00	Water (?)	0.75	2	0.38	>1024	1	1	0.002	1.5	4	12	0.38	0.016	4	0.125
TCD 192729	2/4/2007, 15h00	Water (Christiana)	1	2	0.5	>1024	1	1	0.004	1.5	6	12	0.38	0.016	1.5	0.094
TCD 193005	8/4/2007, 11h00	Water (?)	1	2	0.38	>1024	1	1	0.002	1	8	12	0.38	0.016	1	0.094

*AMP, ampicillin; AUG, augmentin; TRI, trimethoprim; SUL, sulfamethoxazole; CHL, chloramphenicol; NAL, nalidixic acid; CIP, ciprofloxacin; TET, tetracycline; KAN, kanamycin; STR, streptomycin; IMI, imipenem; CRO, ceftriaxone; ERY, erythromycin; CAZ, ceftazidime.

various sections of two major rivers which pass through the Northern Cape province of SA. These ten water strains showed a diversity of PFGE profiles (data not shown) and REP-PCR profiles (Fig. 1). Dendrogram analysis distinguished five clones amongst these ten water strains (data not shown). This high level of genetic diversity was expected and is in agreement with numerous studies which have reported extensive genetic diversity among strains of non-O1, non-O139 *V. cholerae* (Pang *et al.*, 2007). Furthermore, dendrogram analysis differentiated our *V. cholerae* strains from a cluster of Southern African *V. cholerae* O1 strains which included *V. cholerae* O1 from the 1997–2005 SA epidemic, *V. cholerae* O1 from the 1980s SA epidemic and *V. cholerae* O1 from the 2006–2007 Namibian outbreak (data not shown); this provided further evidence for the proposed non-O1 serotype status of the current strains. Most of the *V. cholerae* strains were isolated from water samples taken along the Vaal River. This would not be the first report of *V. cholerae* in the Vaal River. Recently, Le Roux *et al.* (2006) described the presence of a genetically diverse population of *V. cholerae* in the Vaal Barrage. The Vaal Barrage is located far off upstream of the current water sampling sites. Two of our current strains (191499 and 191717) were isolated from water samples taken at the

Vaalharts Weir located on the Vaal River. These two strains showed a PFGE profile (data not shown) and a REP-PCR profile (Fig. 1) which were identical to those of the human strain (190511) and also showed antimicrobial susceptibility profiles similar to that of the human strain (Table 1). These data suggest that the human strain and the strains from the Vaalharts Weir were related. The Vaalharts Weir is a major diversion weir on the Vaal River, from which water is diverted by canals to surrounding areas. The Vaalharts Weir supplies water to the Phokwane municipality (located 44 km away), which in turn serves the local township of Pampierstad, where the patient lived. No information on water quality was available from the Phokwane municipality. The patient lived in a low-cost formal dwelling in an area serviced by a piped municipal water supply. She was unemployed with no recent travel history. Anecdotal evidence suggests that although residents have access to hygienic municipal water, they sometimes still choose to collect water from open canals. Exactly how the patient became infected with *V. cholerae* remains speculative; however, scientific evidence strongly suggests that the Vaalharts Weir was the source of the infecting strain. This study has highlighted the continued presence of *V. cholerae* in the Vaal River of SA. Although these environmental strains

have low outbreak potential, their presence suggests that environmental conditions are suitable for survival of epidemic *V. cholerae* O1 in untreated groundwater, and vigilance regarding water quality must be maintained, even in those provinces of SA which previously have not been affected by a cholera epidemic. Although our patient was not infected by epidemic *V. cholerae* O1, we have shown that in a patient with severe immunosuppression, co-infection with non-epidemic *V. cholerae* and *E. coli* can have a devastating and deadly consequence.

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Fig. 1. REP-PCR fingerprint patterns of *V. cholerae* strains. Lanes: 2, strain 190511; 3, strain 191499; 4, strain 191717; 5, strain 191718; 6, strain 191723; 8, strain 191908; 9, strain 192117; 10, strain 192725; 11, strain 192727; 12, strain 192729; 13, strain 193005; 1, 7 and 14, DNA size standard in base pairs (Bioline HyperLadder I).

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