Surveillance for enterohaemorrhagic *Escherichia coli* associated with human diarrhoea in South Africa, 2006–2009

Enterohaemorrhagic *Escherichia coli* (EHEC) is primarily a foodborne bacterial pathogen that causes outbreaks of disease worldwide (Currie *et al.*, 2007; King *et al.*, 2009). The disease is mostly associated with diarrhoea (often bloody) and abdominal pain; however, EHEC infections can cause severe complications such as haemolytic uraemic syndrome (HUS) (Orth & Würzner, 2006). Very few published data exist on the occurrence of EHEC infections in humans in southern Africa. The first definitive report of EHEC in Africa was in 1990 from South Africa and involved a sporadic case of *E. coli* O157:H7 haemorrhagic colitis (Browning *et al.*, 1990), although Barnard & Kibel (1965) collected a cluster of cases of HUS in children following an episode of diarrhoea in Zimbabwe, before the mechanism of disease had been elucidated. The most notable EHEC publication from southern Africa described an outbreak of bloody diarrhoea in Swaziland and the eastern regions of South Africa in 1992, which involved thousands of cases and was caused by *E. coli* O157; major contributing factors were carriage of *E. coli* O157 by cattle, death of cattle secondary to drought, and heavy rains that resulted in contamination of surface water by dead and dying cattle (Effler *et al.*, 2001; Isaäcson *et al.*, 1993). In the current study, we describe the occurrence of EHEC among human isolates of *E. coli* submitted for extended analysis to an enteric diseases reference laboratory as part of a national surveillance programme in South Africa.

The Enteric Diseases Reference Unit (EDRU) of the National Institute for Communicable Diseases is a reference centre in South Africa for human infections involving enteric pathogens including diarrhoeagenic *E. coli*, *Salmonella* species, *Shigella* species and *Vibrio cholerae*. The EDRU participates in national laboratory-based surveillance for these pathogens. Isolates are voluntarily submitted to the EDRU from ~200 clinical

microbiology laboratories across the country. For the current study, isolates identified as suspected diarrhoeagenic *E. coli* by participating laboratories were submitted to the EDRU, where organism identification was confirmed using standard microbiological identification techniques. Serotyping of *E. coli* strains for O-antigen was performed using the tube agglutination test (antisera manufactured by the Statens Serum Institut, Copenhagen, Denmark) using previously described methods (Orskov *et al.*, 1977). H-antigen serotyping was not undertaken. Susceptibility testing to antibiotics was determined by Etest (bioMérieux). The presence of extended-spectrum β -lactamase (ESBL) activity was investigated by disc diffusion screening methods, using discs manufactured by Mast Group, following methods as described by the Clinical and Laboratory Standards Institute (CLSI, 2005). PCR was used to distinguish diarrhoeagenic *E. coli* strains from non-pathogenic *E. coli* strains. For identification of EHEC, we used PCR targeting the following genes: *eae* (encoding the intimin outer-membrane protein), *hlyA* (encoding enterohaemolysin), *stx1* [encoding Shiga toxin 1 (Stx1)], *stx2* [encoding Shiga toxin 2 (Stx2)] and *uidA* (encoding β -glucuronidase). We used previously described methods for PCR (Vidal *et al.*, 2005; Paton & Paton, 1998; Cebula *et al.*, 1995) and included internationally accepted positive control strains. EHEC was defined by positive PCR results for an *eae* gene and a *stx* gene. Genotypic relatedness of isolates of the same serotype was investigated by PFGE analysis of *Xba*I-digested genomic DNA on a CHEF-DR III electrophoresis system (Bio-Rad Laboratories) using a PulseNet protocol (Ribot *et al.*, 2006).

For the years 2006–2009, the EDRU received 2378 isolates of *E. coli* for extended laboratory analysis; most isolates had been identified as enteropathogenic

E. coli, based on testing with pooled antisera for these pathogens. Only 14 of the 2378 isolates were determined to be EHEC. For information regarding the identification of other classes of diarrhoeagenic *E. coli*, this can be accessed in reports of EDRU surveillance data located at <http://www.nicd.ac.za/?page=germs-sa&id=39>. All EHEC isolates were cultured from stool specimens from patients presenting with diarrhoea. Unfortunately, our *E. coli* isolates are not accompanied by comprehensive clinical details and we do not get the full history of the specimen or the patient. No further information was available regarding any clinical complications (such as HUS) experienced by patients or mortality, due to delays in forwarding the isolates to the EDRU and loss of patients to follow-up. Patient age ranged from 1 to 24 months, with the exception of a single 45-year-old patient (median age=9.5 months) (Table 1). The 14 EHEC isolates revealed a diversity of serotype status (O4, O5, O21, O26, O84, O111 and O157), with serotypes O26 ($n=5$) and O111 ($n=3$) being the most commonly encountered (Table 1). Serotypes O26 and O111 are recognized worldwide as two of the four major non-O157 serotypes (O103, O111, O145 and O26) associated with Shiga toxin-producing *E. coli* (Caprioli *et al.*, 2005). One of our EHEC isolates serotyped as O157 and tested positive for *eae*, *stx2* and *hlyA* genes; it was unable to ferment sorbitol and lacked β -glucuronidase activity (determined through detection of a mutant *uidA* gene). Apart from this single sorbitol non-fermenter, the other 13 EHEC isolates were all able to ferment sorbitol. All but three of our EHEC isolates were positive for Stx1 only, two isolates were positive for Stx2 only and one isolate was positive for both Stx1 and Stx2 (Table 1). The Shiga toxin genotype is strongly associated with the severity of disease: patients infected with EHEC strains

Table 1. Summary of data for EHEC associated with human diarrhoea in South Africa, 2006–2009

Age of patient	Province	Specimen collection date	Strain no.	Serotype	Positive for virulence genes	Antibiotic susceptibility*†												
						Amp	Aug	Tri	Sul	Chl	Nal	Cip	Tet	Kan	Str	Imi	Cro	Caz
2 years	Gauteng	10/01/2006	106336	O111	<i>eae, stx1, stx2, hlyA</i>	S	S	S	S	S	S	S	S	S	S	S	S	S
45 years	Mpumalanga	07/11/2007	229118	O26	<i>eae, stx1, hlyA</i>	R	S	R	R	R	S	S	S	S	R	S	S	S
11 months	Gauteng	05/12/2008	309554	O111	<i>eae, stx1</i>	R	S	R	R	S	S	S	R	S	R	S	S	S
12 months	Gauteng	12/06/2008	268325	O111	<i>eae, stx1</i>	S	S	S	S	R	S	S	S	S	S	S	S	S
15 months	Gauteng	21/08/2008	282634	O21	<i>eae, stx1, hlyA</i>	S	S	S	R	S	S	S	R	S	S	S	S	S
9 months	Gauteng	27/08/2008	282628	O26	<i>eae, stx1, hlyA</i>	S	S	S	S	R	S	S	S	S	S	S	S	S
1 month	Eastern Cape	21/01/2008	240779	O26	<i>eae, stx1, hlyA</i>	R	S	R	R	S	S	S	S	S	R	S	S	S
9 months	Gauteng	25/11/2008	307439	O4	<i>eae, stx1, hlyA</i>	S	S	S	S	S	S	S	S	S	S	S	S	S
9 months	Gauteng	01/11/2008	302692	O5	<i>eae, stx1</i>	S	S	S	S	S	S	S	S	S	S	S	S	S
10 months	Gauteng	30/03/2008	251240	O84	<i>eae, stx2</i>	S	S	S	S	R	S	S	R	S	S	S	S	S
9 months	Gauteng	16/04/2009	339907	Untypable	<i>eae, stx1</i>	R	S	R	R	S	S	S	R	S	S	S	S	S
18 months	Gauteng	10/01/2009	319987	O26	<i>eae, stx1</i>	S	S	S	S	R	S	S	S	S	S	S	S	S
4 months	Gauteng	09/09/2009	377720	O26	<i>eae, stx1</i>	S	S	S	R	S	S	S	S	S	S	S	S	S
20 months	Gauteng	19/11/2009	413642	O157	<i>eae, stx2, hlyA</i>	S	S	S	S	S	S	S	S	S	S	S	S	S

*S, Susceptible; R, resistant.

†Isolates were determined to be antibiotic resistant at the following MIC breakpoints: ampicillin (Amp), MIC $\geq 16 \mu\text{g ml}^{-1}$; augmentin (Aug), MIC $\geq 16 \mu\text{g ml}^{-1}$; trimethoprim (Tri), MIC $\geq 16 \mu\text{g ml}^{-1}$; sulfamethoxazole (Sul), MIC $\geq 512 \mu\text{g ml}^{-1}$; chloramphenicol (Chl), MIC $\geq 16 \mu\text{g ml}^{-1}$; nalidixic acid (Nal), MIC $\geq 32 \mu\text{g ml}^{-1}$; ciprofloxacin (Cip), MIC $\geq 2 \mu\text{g ml}^{-1}$; tetracycline (Tet), MIC $\geq 8 \mu\text{g ml}^{-1}$; kanamycin (Kan), MIC $\geq 32 \mu\text{g ml}^{-1}$; streptomycin (Str), MIC $\geq 64 \mu\text{g ml}^{-1}$; imipenem (Imi), MIC $\geq 8 \mu\text{g ml}^{-1}$; ceftriaxone (Cro), MIC $\geq 16 \mu\text{g ml}^{-1}$; ceftazidime (Caz), MIC $\geq 16 \mu\text{g ml}^{-1}$.

harbouring Stx2 develop HUS significantly more frequently than patients infected with strains harbouring Stx1 (Orth & Würzner, 2006). Susceptibility to commonly used antibiotics varied from isolates with full susceptibility to isolates with resistance to multiple antibiotics (Table 1). None of the EHEC isolates showed ESBL activity. Each of the 14 EHEC isolates showed a unique PFGE pattern (data not shown) indicating that no relationship existed between any of the isolates, irrespective of serotype.

In summary, EHEC is rarely isolated from humans in South Africa and is usually a coincidental finding. These are sporadic infections and aside from the single outbreak in Swaziland and South Africa in 1992, EHEC has not been found to be associated with extensive outbreaks of disease in southern Africa. South Africa appears to follow the interesting phenomenon observed in developing countries wherein EHEC is much less frequently isolated than other classes of diarrhoeagenic *E. coli*, such as enteropathogenic *E. coli*, enterotoxigenic *E. coli* and enteroaggregative *E. coli*. It is difficult to speculate on the reasons for the low incidence and reporting of EHEC from developing countries as the reasons are most likely multifactorial; however, we concur with the belief that cattle farming and feeding practices have a major role to play (Callaway *et al.*, 2009). In terms of human infection, cattle are the major reservoir for EHEC infection worldwide. Developing countries still tend to rely mostly on traditional farming practices where cattle are free-roaming with a diet consisting mainly of naturally growing grass, whereas in developed countries there is an increased practice of rearing cattle in feedlots (factory farming) with specialized diets including grain, hay, corn, sorghum and other by-products of food processing. Studies have shown that diet can affect the carriage and shedding of *E. coli* in cattle (Callaway *et al.*, 2009), which in turn would affect the spread of *E. coli* infection to humans directly through consumption

of animal products or indirectly via environmental contamination of drinking water and plant products grown for human consumption.

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