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Molecular basis of virulence in clinical isolates of *Escherichia coli* and *Salmonella* species from a tertiary hospital in the Eastern Cape, South Africa

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Abstract

Background: Apart from localized gastrointestinal infections, *Escherichia coli* and *Salmonella* species are major causes of systemic disease in both humans and animals. *Salmonella* spp. cause invasive infections such as enteric fever, septicemia, osteomyelitis and meningitis while certain types of *E. coli* can cause systemic infections, including pyelonephritis, meningitis and septicemia. These characteristic requires the involvement of a myriad of virulence factors.

Methods: This study investigated the virulence factors of *Escherichia coli* and *Salmonella* species in clinical specimens from patients with diarrhoea presenting to health care centres in Oliver R. Tambo District Municipality, Eastern Cape Province, Republic of South Africa. Microbiology analysis involved the use of cultural and molecular techniques.

Results: Out of a total of 315 samples screened, *Salmonella* isolates were obtained in 119 (37.8%) of cases and these comprised: *S. choleraesuis* (6%), *S. enteritidis* (4%), *S. eppendorf* (1%), *S. hadar* (1%), *S. isangi* (8%), *S. panama* (1%), *S. typhi* (52%), *S. typhimurium* (25%) and untyped *Salmonella* spp. (2%). Among the *Salmonella* species 87 (73.1%) were invasive. Using molecular diagnostic methods, diarrheagenic *E. coli* were detected in 90 cases (28.6%): the greater proportion of this were enteroaggregative *E. coli* (EAEC) 37 (41.1%), enteropathogenic *E. coli* (EPEC) 21 (23.3%) and enterohemorrhagic *E. coli* (EHEC) 21 (23.3%). The predominant virulence gene among the diarrheagenic *E. coli* was EAEC heat-stable enterotoxin *astA* genes while the virulence genes identified in the *Salmonella* strains were 15 (12.6%) *fliC* and 105 (88.2%) *inv* genes. The amino acid identity of the representative genes showed 95-100% similarity to corresponding blast searched sequence.

Conclusions: This study showed the diversity of virulence gene expression in two major enteric pathogens. *S. typhi* and enteroaggregative *E. coli* were the predominant enteropathogens in our study area with an indication that EAEC is endemic within our study population. It was observed among other things that some diarrheagenic *E. coli* isolated from apparently asymptomatic subjects expressed some virulence genes at frequency as high as seen in diarrheagenic cases. This study underlines the importance of understanding the virulence composition and diversity of pathogens for enhanced clinico-epidemiological monitoring and health care delivery.

Background

Gastrointestinal infections due to pathogenic *Enterobacteriaceae* in particular *Escherichia* and *Salmonella* species are significant causes of morbidity and mortality worldwide. These infections which usually are self-limiting may be fatal in hosts with debilitating immune

systems [1]. The fatality of infections due to these enteric pathogens depends on their serotypes, the size of the inoculum, and the status of the host [2]. *Escherichia* and *Salmonella* species were reported to have diverged from a common ancestor based on the evolutionary rate estimates from 5S and 16S rRNA sequence analyses while *Shigella* spp. are considered clonal lineages of *Escherichia coli* [3]. *Salmonella* species are mainly pathogenic, with differing host ranges. *S. typhi* is adapted to humans and does not occur in animals while

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non-typhoidal *Salmonella* serovars (NTS) have a broad vertebrate host range [2]. Even though *E. coli* is generally known as commensal normal flora of the gut, some *E. coli* strains are the causative agents of neonatal meningitis, urinary tract infections, bacteremia, and infectious diarrhea.

The major distinguishing factor between pathogenic and non-pathogenic strains of *E. coli* strains is the occurrence of virulence genes, which code for the various known strategies for pathogenicity. Analysis have shown that pathogenic *E. coli* strains from diarrhoea cases and those involved in urinary tract infections are more of a distinct subsets of *E. coli*, rather than a reflection of the random fecal flora [4]. Some of the virulence factors of *E. coli* include ability to adhere, colonize, and invade the hosts' cells. Further to these are the secretion systems, production of cell surface molecules, transport and siderophore formation [5]. According to Kaper *et al.*, [6], *E. coli* has been categorized based on the type of virulence factors present and host clinical symptoms basically into the following pathotypes: enteropathogenic *E. coli* (EPEC); enterohemorrhagic *E. coli* (EHEC); enterotoxigenic *E. coli* (ETEC); enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC), a subclass of enteroaggregative *E. coli*; enteroinvasive *E. coli* (EIEC); uropathogenic *E. coli* (UPEC) and neonatal meningitis *E. coli* (NMEC).

The ability of the enteric pathogen to invade and penetrate intestinal epithelial cells is required in salmonellosis whether it is confined as the intestinal form or progresses to systemic involvement [7]. The attribute to direct their internalization by the epithelial cells which are not normally phagocytic is a striking *Salmonella*-host cell interaction. According to Galan and Curtiss [8] this remarkable phenotype known as invasion allowed for identification and characterization of invasion genes. The key mechanism involves type III secretion systems which are encoded by pathogenicity island 1 (SPI-1) [9]. *Salmonella* also possess the ability to alter phagocytosis in order to circumvent the process. *S. enterica* serovar Typhimurium is known to delay significantly the fusion of the phagosome to the lysosome [10]; thereby hibernating in phagocytic cells and hence adapt to resist the antimicrobial activity of the fused phagolysosome [11]. Bacterial survival in phagocytic cells has been observed as an alternate to invasion in accessing privileged sites in hosts. Rescigno *et al.*, [12] postulated that CD18+ expressing phagocytes are alternate route and these cells have been observed by Vasquez-Torres *et al.*, [13] as vehicles for reaching the spleen in an invasion-independent manner by *S. enterica* serovar Typhimurium.

Molecular analysis is known to give a better picture of epidemiology of infectious diseases. Studies have demonstrated the sensitivities of molecular-based

methods to be greater compared to current conventional methods of analysis [1]. Molecular studies have led to the understanding of the genomic make-up of bacteria which generally consist of stable regions and variable regions, the flexible part that is composed of bacteriophages, plasmids, transposons as well as unstable large regions, called genomic islands. The so-called genomic islands are a gene pool required for encoding virulence factors of pathogenic bacteria and these have been designated "pathogenicity islands" [14]. The concept of pathogenicity islands (Pais) was first identified through the genetic and molecular analysis of virulence genes in uropathogenic *E. coli* and EPEC [15]. Pais which are specific regions of chromosomal DNA have been described in more than 30 bacterial species [14]. It is a well known concept that bacterial pathogenicity is an organized multifactorial process involving numerous chromosomal and extrachromosomal genes directed by complex regulatory circuits [16,17].

There are various shared genetic strategies for pathogenicity in enteric bacilli. Type III secretion is a dedicated secretion machinery whose components are coded for by numerous homologous gene sequences shared by enteric pathogens [3,18]. Nevertheless, there has been understanding that the similarities between EPEC virulence attributes and *Salmonella* invasion genes are more than homologous genes associated with secretion [19]. Most virulence factors of pathogenic *E. coli*, *Shigella*, and *Salmonella* strains are plasmid-borne however; one or more of the essential virulence determinants are borne on an extrachromosomal element [20]. In both *E. coli* and *Salmonella* spp. fimbriae might play a role in adhesion and invasion [21]. The curli fimbriae of these strains were proven to bind to several tissue-matrix proteins as well as plasminogen and its activator t-PA [3].

Bacteria are emerging with new means of circumventing human efforts at curbing their nefarious schemes and various evolution patterns and innovations are certainly put in place by these pathogens. A myriad combination of virulence genes against indiscriminate genetic transfer and recombination are required for a successful emergence of pathogen [22,23]. Profiling the expression of these genes will give impetus to understanding the mechanisms by which enteric bacterial pathogens colonize, spread and at times persist in the hosts [24]. This study investigated the genetic determinants of virulence in *E. coli* and *Salmonella* spp. which are significant pathogens involved in enteric diseases.

Methods

Specimens' collection and bacterial isolation

The study is a retrospective and cross-sectional study which utilized data originally collected for surveillance purposes focusing on Nelson Mandela Academic

Hospital Complex (NAMHC), Mthatha, a health facility which serve as a referral hospital in the Eastern Cape Province of South Africa. Specimens' collection from cases was from both male and female diarrhoeic patients from all age categories and was based on availability. Written informed consent was obtained from all patients, parents or guardians as the case may be and questionnaire was administered by trained volunteer health workers. Information provided include the frequency of episodes of diarrhoea and whether or not antibiotic or other forms of medication has been used. The control specimens were then selected from the group which had not had diarrhea or antibiotic therapy in the preceding 2 weeks. The study protocol and data handling were approved by the WSU ethics committee (Protocol No. 0003/08) as well as the Department of Health, Eastern Cape, South Africa.

Salmonella isolates deposited at the NICD, Johannesburg under the surveillance study of 2005 to 2008 from this tertiary health facility were obtained. For 2009 fresh stool samples in sterile stool jar or rectal swabs in Cary-Blair transport medium per patient were collected from 125 patients presenting with acute diarrhoea in the tertiary referral facility and surrounding clinics and 75 apparently healthy school pupils in three different schools within ORTDM. These were transported on ice pack to the laboratory where analysis was done within 24 hours. Where this is not possible specimen were preserved at temperature between 4 to 8°C.

Bacteriological analyses

NICD isolates

Bacteriological analyses of the specimens for these isolates were carried out at the National Health Laboratory Services, Nelson Mandela Academic Hospital, Mthatha. Samples were examined for the presence of *E. coli*, *Salmonella* and *Shigella* using standard conventional methods according to Forbes *et al.*, [25].

E. coli

The faecal samples were cultivated on MacConkey agar. After overnight incubation at 37°C, lactose fermenting colonies (LFC) with the typical appearance of *E. coli* were selected for further analysis. Isolates were identified by biochemical assays using Microscan Gram negative combo panel NUC 45 (Siemens/Dade Behring).

Salmonella and *Shigella*

Specimens were cultivated for the isolation of *Salmonella* and *Shigella* species on MacConkey agar. After 24 h of incubation at 37°C, suspected colonies with typical characteristics of *Salmonella* and *Shigella* were sub-cultured on XLD (xylose lysine deoxycholate) agar for 24 h at 37°C. Confirmation was carried out using Microscan Gram negative combo panel NUC 45 (Siemens/Dade Behring).

DNA extraction

DNA template for PCR was obtained from pure overnight bacterial culture using Fungal/Bacterial DNA extraction kit™ (Zymo Research) and following manufacturer's instructions. The concentration of the eluted DNA was measured using NanoDrop 2000 spectrophotometer (Thermo Scientific).

DNA amplification

PCR amplifications were performed in a final volume of 25 µl containing: 0.5 to 2 µl of DNA template depending on concentration, 8.5 to 10 µl of Nuclease free water, 1 µl of each primer and 12.5 µl Master mix (EconoTaq Green, Fermentas). Amplifications were carried out in a GeneAmp PCR System 9700 Thermocycler (Applied Biosystems). All oligonucleotide primers were synthesized by Inqaba Biotechnology (Pretoria, South Africa) and the sequences are as shown in Table 1 (63-72). The PCR cycling conditions for the *E. coli* strains consisted of 95°C for 5 min while for the *Salmonella* isolates consisted of 95°C for 1 min, which were followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and elongation at 72°C for 30 s. Amplification products were separated by electrophoreses on 10 mg/ml agarose gel (TopVision™, Fermentas) in 1× TBE Buffer and ethidium bromide (5 µl) with a 100-bp ladder (Fermentas) as molecular weight marker.

Sequencing reaction

PCR products were sequenced using an Applied Biosystems 3500xL Genetic analyzer (AB Biosystems). Prior to PCR products sequencing, the unincorporated dNTPs were dephosphorylated with a commercial kit from Zymo Research Corporation (Orange, CA). Subsequently, the PCR products were sequenced with the ABI PRISM BigDye terminator cycle sequencing ready reaction kit (AB Biosystems) using the same primers as employed in the PCR reactions. The products were then subjected to the following conditions: 94°C for 2 min, followed by 40 cycles of denaturation at 85°C for 1 s, annealing at 53°C for 10 s and extension at 60°C for 2 min 30 s, with a final extension at 4°C for 0 s. The sequencing reaction products were cleaned up using ZR-96 DNA sequencing clean-up kit™. Thereafter, the ultra-pure products were analyzed on the sequencing machine. Sequences were aligned with known *E. coli* and *Salmonella* virulence gene sequences by a blast search of the National Center for Biotechnology Information (NCBI) data base <http://www.ncbi.nlm.nih.gov/BLAST/> using Staden package version 1.6.0-beta4 (MRC.WTSI).

Results

Demographic features

Patients' data were analyzed using Microsoft Excel version 2003. Continuous variables were summarized as

Table 1 Primer sets for the pathotypes and virulence genes for the *E. coli* and *Salmonella* spp

Isolate species/subgroups	Target gene	Primer	Nucleotide Sequence (5'- 3')	Amplicon size (bp)	Reference		
<i>E. coli</i>							
EPEC and EHEC	<i>eaeA</i>	EAE-a	ATGCTTAGTGCTGGTTTAGG	248	[63]		
		EAE-b	GCCTTCATCATTTTCGCTTTC				
EHEC	<i>stx1</i>	JMS1-F	GTCACAGTAACAAACCGTAACA	95	[64]		
		JMS1-R	TCGTTGACTACTTCTTATCTGGA				
ETEC	LT	LT-1	AGCAGGTTTCCCACCGGATCACCA	132	[65]		
		LT-2	GTGCTCAGATTCTGGGTCTC	190	[66]		
EAEC	<i>aggR</i>	STa-F	GCTAATGTTGGCAATTTTATTCTGTA	254	[67]		
		STa-R	AGGATTACAACAAAGTTCACAGCAGTAA				
		AggRks1	GTATACACAAAAGAAGGAAGC			106	[68]
		AggRks2	ACAGAATCGTCAGCATCAGC				
EIEC	<i>astA</i>	EAST-1S	GCCATCAACACAGTATATCC	215	[69]		
		EAST-1AS	GAGTGACGGCTTTGTAGTCC				
		VirA-F	CTGCATTCTGGCAATCTCTTCACA				
		VirA-R	TGATGAGCTAACTTCGTAAGCCCTCC				
<i>Salmonella</i>							
	<i>invA</i>	invA 139	GTGAAATATTCGCCACGTTCCGGGCAA	284	[70]		
		invA 141	TCATCGCACCGTCAAAGGAACC				
	<i>sefA</i>	S1	GCC GTA CAC GAG CTT ATA GA	250	[71]		
		S4	ACC TAC AGG GGC ACA ATA AC				
	<i>fliC</i>	Fli15	CGG TGT TGC CCA GGT TGG TAA T	620	[72]		
		Typ04	ACT GGT AAA GAT GGC T				

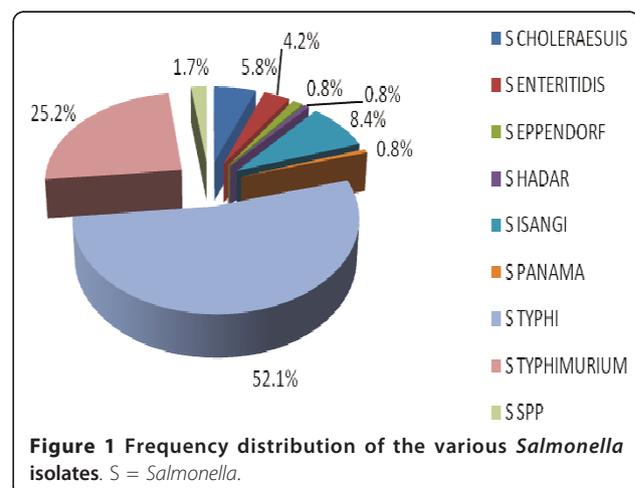
fliC-flagellin H1; *invA*-invasion; *sefA*- fimbrial antigen; *aggR*- transcriptional activator for EAEC aggregative adherence fimbria I expression; *eaeA*-*E. coli* attaching and effacing; *astA*-EAEC heat-stable enterotoxin; LT- heat-labile enterotoxin; ST- heat-stable enterotoxin; *VirA*-virulence plasmid.

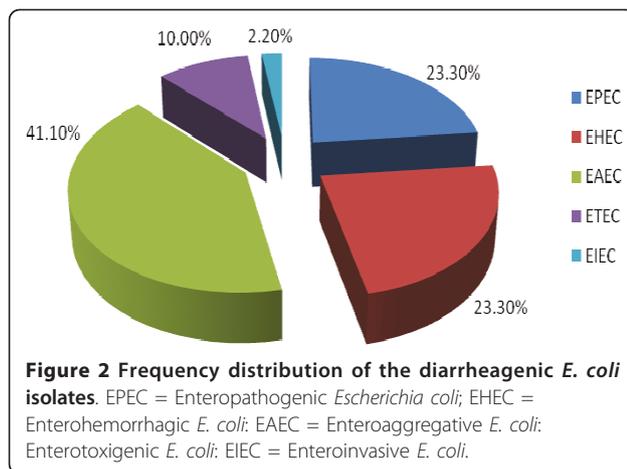
mean. Our subjects were predominated by male patients 165/315 (52.4%) and no age group was excluded, with the youngest patient being 3 months and the oldest 91 years. A sizeable number of cases, 95 (30.15%) were between the ages 7 to 13 and controls school-aged 7 to 12 were matched for this age category.

Bacteriological identification and Molecular analysis

Results showed that *Salmonella* strains were isolated from 119 (37.8%) of cases while diarrheagenic *E. coli* was found in 90 (28.6%) of cases. The distributions of the different pathotypes are as shown in Figures 1 and 2. Of the *Salmonella* isolates 87 (74.1%) were invasive. The most common virulence factors detected among the *Salmonella* strains were *invA* found in 105 *Salmonella* spp. and *fliC* genes detected in 15 *Salmonella* isolates. The predominant virulence gene among the diarrheagenic *E. coli* was 24 EAEC heat-stable enterotoxin *astA* genes. Table 2 showed the distribution of the various genes among cases and controls. The representative gels for PCR amplification of DNA extracted from selected *E. coli* and *Salmonella* isolates showing the presence of diverse virulence genes are indicated in additional files 1A, B and 2 respectively. One hundred and eighty isolates were obtained from the 150 control

subjects. *E. coli* was the predominant bacterial species being 85 (47.2%) while *Salmonella* spp. was 8 (12.1%). Other recovered bacteria species were *Proteus mirabilis* 45 (25.0%), *Klebsiella pneumoniae* 23 (12.8%) and *Enterobacter cloacae* 19 (10.5%). The sequencing analysis of our genes showed 100% conformation of the various virulence genes with corresponding blast search sequence and confirmed the strain.





Discussion

Gastroenteritis is a major concern in sub-Saharan Africa as with other developing countries [26]. South African National Burden of Disease study of the year 2000 found that diarrhoea accounted for nearly 3% of all deaths in South Africa [27]. According to the South African health review of 2007, death due to gastroenteritis among children was put at 15% [28] showing increasing mortality. The developed countries are not spared in the global burden of enteric-related diarrhoea. Salmonellosis was considered a major public health problem in the United States [29]. *E. coli* and *Salmonella* are among the bacterial pathogens implicated in gastroenteritis. These enteric pathogens have evolved different strategies for subverting normal host cellular functions [30]. These pathogens cause various intestinal and extraintestinal diseases by means of virulence factors that affect a wide range of cellular processes. These virulence induced infections usually involve complex mechanisms with various specific, interdependent interactions between hosts and pathogens [31]. This present study provides information on the pathotypes and some virulence factors associated with local isolates of *E. coli* and *Salmonella* species. *E. coli* are more than just a harmless intestinal microflora; it can also be a highly versatile, and frequently deadly, pathogen [6]. *E. coli* strains cause diarrhoea by several distinct pathogenic mechanisms and differ in their epidemiology. Virulence genes were

expressed in diarrheagenic *E. coli* from both cases and controls. EAEC was detected in 37 (41.1%) cases involving diarrhoeagenic *E. coli*. Studies conducted in Thailand and Brazil, reported a frequency of 12% and 11% EAEC respectively among children with acute diarrhoea [32,33]. Although the prevalence of EAEC is believed to be considerably higher in the developing countries compared to industrially developed countries, a Switzerland study, reported that EAEC was encountered in a significant proportion of diarrhoea cases among children [34]. As evident in this study and previous studies, EAEC seems to be endemic within our study population and other locations in sub-Saharan Africa [35,36], emerging as a significant diarrheal agent worldwide with the pattern of infection changing from persistent diarrhoea to include acute diarrhoea [37].

The second diarrheagenic *E. coli* type detected was EHEC constituting 21 (23.3%) of all diarrheagenic *E. coli*. Enterohaemorrhagic *E. coli* (EHEC) is a subset of Shiga toxin-producing *Escherichia coli* (STEC) which is associated with severe systemic disease as haemorrhagic colitis, haemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura, particularly in infants, young children and in the elderly [38,39]. EHEC infects the large bowel and inflict damage to the colon with infectious dose estimated to be less than 100 CFU [40]. Of the various virulence factors associated with pathogenicity in the EHEC strain, this study failed to detect both Shiga toxin 1 (*stx1*) and Shiga toxin 2 (*stx2*) but only detected intimin which is encoded by the *eaeA* gene [41]. Intimin is known to facilitate the adherence of pathogen to intestinal villi producing attaching and effacing lesions [42]. Previous studies have implicated EHEC in outbreaks and sporadic infections both in the United States and around the world [43,44].

Intimin (*eaeA*) gene was also detected in 21 (23.3%) of EPEC. Unlike most studies where ETEC often take the lead in bacterial enteritis due to *E. coli*, a study by Weggerhof [45] also reported a higher incidence of EPEC from the screening of some pediatric patients with diarrhoea in Mpumalanga Province of South Africa. More recent studies described the contributions of EPEC to the human disease burden as significant [6,46]. Thus EPEC plays a vital role in acute diarrhoea. EPEC is

Table 2 Distribution of virulence genes among the *E. coli* and *Salmonella* spp

Bacterial Strain	Number of isolate	Virulence genes								
		<i>fliC</i>	<i>invA</i>	<i>sefA</i>	<i>aggR</i>	<i>eaeA</i>	<i>EAST</i>	<i>LT</i>	<i>ST</i>	<i>virA</i>
<i>Salmonella</i> spp. (case)	119	15	105	0	ND	ND	ND	ND	ND	ND
<i>Salmonella</i> (control)	8	0	7	0	ND	ND	ND	ND	ND	ND
<i>E. coli</i> (case)	90	ND	ND	ND	13	21	24	5	0	2
<i>E. coli</i> (control)	85	ND	ND	ND	10	11	19	0	0	0

ND-Not determined.

known to cause illness manifesting as watery diarrhoea with little inflammation of the intestinal mucosa [47]. Virulence is initiated in EPEC by the induction of a characteristic ultrastructural lesion in which the bacteria make intimate contact with the apical plasma membrane, causing localized destruction of the intestinal brush border and distortion of the apical enterocyte membrane [48] as is in the classical attaching and effacing (AE) lesion.

The ETEC and EIEC strains were found only in 9 (10.0%) and 2 (2.2%) of cases with diarrheagenic *E. coli* respectively. Although ETEC strains have been described as a major contributor to infantile diarrhoea in developing countries and of travellers' diarrhoea in visitors to these countries [49,50], our findings were different showing a decline in the involvement of these strains in our setting. ETEC strains cause secretory diarrhoea similar to that of *Vibrio cholerae* by forming plasmid encoded heat-labile (LT) or heat-stable (ST) enterotoxins genes [47,51]. ETEC engage strain-specific antigenic, hair-like fimbriae in attachment to specific receptors on the surface of enterocytes in the intestinal lumen [50]. EIEC on the other hand produce dysentery-like diarrhoea similar to that caused by *Shigella* species by invading and multiplying within epithelial cells of the colonic mucosa, resulting in an intense inflammatory response characterized by abscesses and ulcerations that damage the integrity of the epithelial cell lining of the colon [52]. EIEC was not a major enteric bacterial pathogen observed in this study, the prevalence was the least (2.2%) and this was similar to that obtained in the study conducted in Mexico City by Paniagu *et al.*, [53] where EIEC was the least detected in the patient group (1%). This pattern is not consistent with studies in other developing countries where EIEC strains were important causes of pediatric diarrhea and dysentery [54,55].

Salmonella species are an important cause of varying food and water-related infections. This study detected *Salmonella* as a major cause of gastroenteritis in our setting. *Salmonella* has previously been described as one of the common causes of gastroenteritis particularly in the developing countries [56,57]. On the contrary, infectious diarrhoea in the developed world is often due to viruses [58]. The most common species isolated in this study were *S. typhi* (52%) and *S. enterica* serovar Typhimurium (25%). This report is consistent with other studies conducted in Iran and South Africa where *S. typhi* and *S. enterica* serovar Typhimurium were described as major aetiological agents of infectious diarrhoea [58,59]. *S. enterica* serovar Isangi was third in ranking of frequency of isolation. Kruger *et al.*, [60] described the increasing importance of this serotype of non-typhoidal *Salmonella* (NTS) which was a rare serotype in South Africa until 2002. Other species identified were *S.*

enterica serovar Choleraesuis, *S. enterica* serovar Enteritidis, *S. enterica* serovar Eppendorf, *S. enterica* serovar Hadar, *S. enterica* serovar Panama and untyped *Salmonella* spp. The virulence factor detected among the majority (105) of the *Salmonella* spp. was *invA*. This gene which is chromosomally located aids attachment of the pathogen to the epithelial cells [8]. The other detectable virulent gene was *fliC* detected in 15 isolates. The flagellin gene, *fliC* is known to aid systemic spread of pathogen and is specific for *S. enterica* serovar Typhimurium [61]. Enteric bacteria possessing *sefA* which encodes the SEF14 fimbrial antigen, a virulence plasmid specific for *S. enterica* serovar Enteritidis [62] were not encountered in this study.

Conclusions

This study showed the diversity of virulence gene expression in two major enteric pathogens. It was observed among other things that some diarrhoeagenic *E. coli* isolated from apparently asymptomatic subjects expressed some virulence genes at frequency as high as seen in diarrhoeagenic cases. This is a pointer to the fact that asymptomatic individuals serve as reservoirs of pathogenic strains of enteric bacteria and may play a role in the spread and acquisition of virulence genes.

Additional material

Additional file 1: Representative gels for PCR amplification of DNA extracted from selected *E. coli* isolates showing the presence of diverse virulence genes. A: 100 bp molecular weight marker (lanes 1 and 10), fragment from *aggR* (lanes 2 to 4), *eeaeA* (lanes 5 to 6) and *astA* (lanes 7 to 9). B: 100 bp molecular weight marker (lanes 1 and 6), fragment from *aggR* (lanes 2 to 3), *virA* (lanes 4) and LT (lanes 5). The relative positions in the gel of predicted size of PCR products are indicated by arrowheads on the right sides.

Additional file 2: Representative gels for PCR amplification of DNA extracted from selected *Salmonella* isolates showing the presence of diverse virulence genes. 100 bp molecular weight marker (lanes 1 and 8) *fliC* (lanes 2 to 4) and *invA* (lanes 5 to 7). The relative positions in the gel of predicted size of PCR products are indicated by arrowheads on the right sides. The relative positions in the gel of predicted size of PCR products are indicated by arrowheads on the right sides.

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Authors' contributions

MAB participated in the design of the study, carried out laboratory analysis and drafted the manuscript. CLO conceived of the study, participated in the design and coordination of the study, supervised the study and revised the manuscript. TH coordinated bench work between collaborators in South Africa and Japan and helped to revise the manuscript. KAB was involved in coordination and facilitated activities at NICD. SDV assisted with the concept and design of the study. Authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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