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ORIGINAL RESEARCH

Molecular detection of zoonotic pathogens causing gastroenteritis in humans: *Salmonella* spp., *Shigella* spp. and *Escherichia coli* isolated from *Rattus* species inhabiting chicken farms in North West Province, South Africa

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Rodents are key carriers and reservoirs of various pathogens of public health importance to both human and animal diseases. This research was carried out in order to identify the selected pathogens, namely, *Shigella* spp., *Salmonella* spp. and *Escherichia coli* from rats that inhabit the poultry houses. A total of 154 samples from captured rats were examined for the zoonotic bacterial pathogens, of which 3.3%, 29.9% and 20.7% were harbouring *Shigella* spp., *Salmonella* spp., and *E. coli,* respectively. A total of 14 *Shigella* isolates expressed presence of *ipa*H gene, of which eight and five were positive for *S. sonnei* and *S. boydii*, respectively. For *Salmonella,* 68 isolates were positive for *inv*A and other genes including *spy* with 26 (38%), *sdf*I 2 (18%), *spv*C 14 (20%), *hil*A 28 (41%), *mis*L 43 (63%), *orf*L 31 (46%) and *spi*C 38 (56%). For *E. coli*, the *aggR* gene was the most prevalent (62 [42%]), followed by the *eae* gene, which was only detected in 21 (14%) isolates, while *stx* gene was not detected in any of the samples. This study shows that zoonotic pathogens with virulence genes are circulating in rodents from selected chicken farms in the North West Province of South Africa. Rodents must therefore be regarded as important carriers of zoonotic pathogens that can potentially infect both humans and animals.

Keywords: poultry farms, *Rattus* species, *Salmonella*, *Shigella*, *Escherichia coli*, zoonotic pathogens

Introduction

Rodents are the main carriers and hosts of many important zoonotic pathogens in humans and animals (El-Sharkawy et al. 2017). It is generally known that they play a role in the transmission and spread of human diseases. They mostly eat feed in storage places and leave their excreta behind leading to the spread of pathogenic organisms (Franssen et al. 2016; Jemilehin et al. 2016). Others carry ectoparasites that are vectors of important pathogens of animals and humans. The selected bacteria, that is, *Escherichia coli*, *Shigella* spp. and *Salmonella* spp. for this investigation are of particular public concern as they are known to cause serious diseases in human beings (Hong et al. 2018; Wang et al. 2015).

Salmonella has been isolated from captured rats and mice at poultry farms in several studies elsewhere and is linked to human and animal diseases (Nkogwe et al. 2011; Li et al. 2018; Umali et al. 2012). Rodents have been shown to play a vital role in spreading disease and thus are regarded as important indicator species for disease outbreaks (Jemilehin et al. 2016; Lapuz et al. 2008; López et al. 2012).

Shigellosis is an acute invasive human intestinal infection caused by the bacteria of the *Shigella* genus. (Mokhtari et al. 2012). There are four species in the *Shigella* genus, which include *S. dysenteriae* (serogroup A), *S. flexneri* (serogroup B), *S. sonnei* (serogroup C) and *S. boydii* (serogroup D) (Theron et al. 2001). *Rattus* spp. are known vectors and reservoirs of this pathogen and rabbits, calves, and monkeys have been reported as hosts (Jianjun 2005; Maurelli et al. 1998; Pan et al. 2006).

Escherichia coli is an enterobacterial zoonosis that can be transmitted by rodents, and rats have been implicated in the transmission of this zoonotic pathogen in previous studies (Caballero et al. 2015; Nkogwe et al. 2011). *E. coli* has also been isolated from several animals, birds, reptiles, bats, and wild deer (Adesiyun et al. 1999; Gopee et al. 2000). All these vectors or reservoirs of the pathogen have been linked to infections in human beings. Based on its virulence, this species is classified into five classes; Enteroaggregative *E. coli* (EAEC), Shiga toxin-producing *E. coli* (STEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), attaching and effacing *E. coli* (AEEC) and enteroinvasive *E. coli* (EIEC) (Vendramin et al. 2014).

This study was conducted in order to isolate and identify *Shigella* spp., *Salmonella* spp. and *E. coli* from rats that are found in poultry houses as a way of understanding the potential risk of infections.

Materials and methods

Study area, sampling and rodent identification

The study was conducted in the North West Province of South Africa, which is located between 25 and 28 degrees south of the equator and 22 to 28 degrees longitude east of the Greenwich Meridian. The temperature in the region ranges from 17 °C to 31 °C in summer and 3 °C to 21 °C in winter (Ramatla et al. 2017). A total of 154 rat samples were collected from six randomly selected commercial farms, transported to the laboratory and were

euthanised. Rats were dissected following a procedure described previously (Lapuz et al. 2008; Ramatla 2019) whereby kidney and caecal samples were removed. For rodent identification, DNA was extracted from kidney tissue using QIAamp DNA Blood and Tissue Kit (Qiagen, Hilden, Germany) and then polymerase chain reaction (PCR) was conducted for amplification of *cytochrome oxidase subunit I* gene (*COI*) as reported by Ramatla et al. (2019).

Isolation and identification of bacterial species

Shigella spp.

Shigella was isolated according to the method described by Phiri et al. (2021). Tryptic soy broth (TSB) 10 ml (Oxoid, Basingstoke, UK), was added into the faecal samples collected from caecum which were homogenised by vortexing for two minutes followed by incubation at 37 °C ± 1 °C for 24 hours. Xylose lysine deoxycholate agar (XLD) (Merck, Wadeville, South Africa) was used to increase the chances of culturing and isolation of the *Shigella* spp. The plates were incubated at 37 °C ± 1 °C for 24 hours in an overturned position. Pinkish to reddish colonies were selected and subjected to Gram staining.

Salmonella spp.

Salmonella was isolated according to the modified version of the International Organization for Standardization (ISO-6579: 2002) method as described previously by Mainar-Jaime et al. (2013). Briefly, samples were first pre-enriched in buffered peptone water (BPW) (Oxoid, Biolab, South Africa), then transferred to Mueller-Kauffmann Tetrathionate Novobiocin (MKTTn) broth (Sigma-aldrich, S.A. Barcelona, Spain). Rappaport-Vassiliadis medium with soya (RVS) broth was used to complete the enrichment process (Sigma-Aldrich, S.A. India). Xylose lysine deoxycholate (XLD) agar (Merck, Wadeville, South Africa) and brilliant green agar (BGA) were used to streak loopfuls of the RVS broth-enriched cultures separately onto two selective agar plates, namely: BGA (Scharlau Chemie S.A. Barcelona, Spain) and XLD agar (Merck, Wadeville, South Africa), and they were incubated for 24 hours in an inverted posture at 37 °C ± 1 °C. On XLD agar, two to three red colonies with or without black centres, colourless or opaque-white colonies surrounded by pink or red zones, and red colonies on BGA were recognised as *Salmonella* suspects. These putative colonies were purified by incubation at 37 °C for 24 hours on nutrient agar (NA) (Merck, Wadeville, South Africa).

E. coli

Isolation of *E. coli* from the samples was done following a previously described enrichment method (Caballero et al. 2015) with some modifications. A weight of 5 g of samples was vortexed vigorously in 10 ml of TSB (Oxoid, UK) and incubated at 37 °C for 24 hours. The broth culture was subcultured onto MacConkey's agar (Biolab, supplied by Merck, Johannesburg, South Africa) then incubated at 37 °C for 24 hours. Following incubation, two to three rose pink colonies were selected and sub-cultured on NA (Merck, Wadeville, South Africa), which was then incubated at 37 °C for 24 hours.

Biochemical tests

Shigella spp.

The Gram-negative isolates were subjected to biochemical tests as described by Mokhtari et al. (2012). For the Indole test, the formation of a red ring indicated a positive reaction and were picked for further tests, while a yellow-brown ring indicated a negative reaction. Colonies of the presumptive *Shigella* were added into the urea broth (Biolab, Merck, South Africa) and incubated. The positive isolates of *Shigella* did not show any changes in colour. Furthermore, isolates were also subjected to triple sugar iron (TSI) agar (Biolab, Merck, South Africa) to test for glucose, lactose, and sucrose fermentation. Bergey's manual of systematic bacteriology was used as an identification aid.

Salmonella spp.

All colonies showing rod-shaped Gram-negative bacteria were subjected to biochemical testing as described by Samaxa et al. (2012). *Salmonella* spp. was subjected to catalase, hydrogen peroxide. The presence of enzyme in bacteria is evident with rapid production of gas (oxygen) bubbles and this is considered as indicating the presence of *Salmonella* spp. The isolates were also subjected to the Oxidase test; the isolates which did not show any colour change after smearing the colony over Microbact oxidase strips (MBO266A, Oxoid Ltd., Basingstoke, England) were suspected to be *Salmonella*. Suspected colonies were tested for Indole by using three to five drops of Kovac's reagent (Pro-LAB Diagnostics, IVD, Bromborough, Wirral, UK), whereby yellow or no colour was an indication of suspected *Salmonella*.

E. coli

The *E. coli* isolates were then subjected to Gram-staining. All colonies showing the Gram-negative rod-shape were subjected to biochemical tests, whereby the isolates which were catalase-positive, indole-positive and Voges–Proskauer-negative were considered as *E. coli* strains (Dobrowsky et al. 2014).

Haemolysis test for *E. coli*

Isolates that satisfied morphological structures and primary biochemical tests were plated on blood agar plates and incubated aerobically at 37 °C for 24 hours. *E. coli* specifically appears as mucoid with haemolysis on blood agar plates as described by March and Ratnam (1986).

Molecular characterisation of bacterial isolates

Pure colonies of suspected *Shigella, Salmonella*, and *E. coli* were used to obtain genomic DNA coli cultures using the Fungal/Bacterial Soil Microbe DNA Mini Prep kit (Zymo-Research Fungal/Bacterial Soil Microbe DNA Mini Prep kit, USA) according to the manufacturer's instructions. Extracted DNA was eluted in a clean 1.5 ml micro-centrifuge tube with 100 ml of DNA elution buffer. The existence of any contaminants was checked using the NanoDrop ND-1000 UV spectrophotometer (Thermo-Fisher Scientific Inc., USA) by measuring the optical density at 280 nm and 260 nm. The isolated DNA was kept at 20 °C until the PCR was done.

Polymerase chain reaction

The final reaction mixture for all PCR assays for *E. coli*, *Shigella* and *Salmonella* in this study was 25 µl and consisted of 8.5 µl double-distilled water, 2 µl of template DNA, the primer mix contained 1 µm of each primer, 2X Dream *Taq* Green PCR Master Mix (2X Dream *Taq* Green buffer, 4 mM $MgCl_2$, 0.4 mM of each dNTP and 1 unit/µl of thermo stable *Taq* polymerase) (Thermo Scientific, USA). The amplification conditions were as follows: 94 °C for 30 sec, 30 cycles at 94 °C for 45 sec, 68 °C for 1 min, followed by 5 min final extension at 68 °C and specific annealing temperatures for each primer are shown in Tables I, II, and III. Standard reference strains were used as a positive control in this study (*Shigella sonnei* ATCC® 25931, *Shigella boydii* ATCC® 9207, and *Shigella flexneri* ATCC® 12022, *Salmonella* Typhimurium ATCC® 14028 and *Escherichia coli* ATCC® 25922) and nuclease-free water was used as a negative control. Amplified fragments of DNA were fractionated on a 2% w/v agarose gel in 0.5 × TAE buffer and visualised under UV light after staining with ethidium bromide (Ekwanzala et al. 2017; Ramatla 2019). To allow standardisation, the 1-kb and 100-bp reference markers (Sigma, D7058) were utilised.

Detection of the *Shigella* spp.

PCR was conducted for amplification of the invasion plasmid antigen H (*ipa*H) gene including three sets of primers to amplify the target genes of *S. boydii* (Conserved Hypothetical Protein), *S. sonnei* (Putative Restriction Endonuclease) and *S. flexneri* (*rfc/wzy*). A method described by Nave et al. (2016) and Alizadeh-Hesar et al. (2014) was used to perform PCR, and primer sequences used are shown in Table I.

Detection of the *Salmonella* species

Eleven genes namely: *fli*C, *spi*C, *mis*L, *Ppb23, spy, spv*C, *orf*L, *inv*A, *sdf I*, *hil*A, and *fli*B were investigated by PCR. *Salmonella* was confirmed using the *inv*A gene, and the virulence of the isolates was determined using the other genes (Table II). Five microlitres of sterile nuclease-free water was used as negative control.

Table I: Sequences of primers, annealing temperatures and amplicon sizes for *Shigella* spp.

Primers	Primer sequence	Repeat size	Annealing temperature (°C)	Species	Reference
ipaH *ipaH*	CGGTCAGCCACCCTCTGAG CTTGACCGCCTTTCCGATACC	613bp	56	*Shigella* spp.	Nave et al. 2016
B-F B-R	TCTGATGTCACTCTTTGCGAGT GAATCCGGTACCCGTAAGGT	248bp	56	*S. boydii*	Ranjbar et al. 2014
Sson-F *Sson*-R	TCT GAATATGCCCTCTACGCT GACAGAGCCCGAAGAACCG	430bp	57	*S. sonnei*	Alizadeh-Hesar et al. 2014
F-F F-R	ACCGGTTATGAACCCTCCAT TGGTGCTTGTTGAGCAACTC	314bp	60	*S. flexneri*	Ranjbar et al. 2014

Table II: List of primers and annealing temperatures used for *Salmonella* spp.

Target gene	Primers	Sequence (5′–3′)	Annealing temperature (°C)	Reference
*inv*A	*Inv*A-F *Inv*A-R	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	58	Ramatla 2019
sdfI	*SdfI*-F *SdfI*-R	TGTGTTTTATCTGATGCAAGAGG TGAACTACGTTCGTTCTTCTGG	57	Ramatla et al. 2020
spy	*Spy*-F *Spy*-R	TTGTTCACTTTTTACCCCTGAA CCCTGACAGCCGTTAGATATT	57	Alvarez et al. 2004; Ramatla et al. 2020
SPAB_01124	*SPAB_01124* -F *SPAB_01124*-R	ACATAATGCTTTTCGTGCTCCTC GGCATAAATATCTTTCTCCCCTCC	60	Ramatla et al. 2020; Zhai et al. 2014
fljB	*FljB*-F *FljB*-R	ATCAACGGTAACTTCATATTTG GGCAACCCGACAGTAACTGGCGATC	60	Perera & Murray 2008; Ramatla 2019
fliC	*FliC*-F *FliC*-R	CACTGGTCTTAATGATGCAGCTC CCTGTCACTTTCGTGGTTAT	60	Ateba & Mochaiwa 2014; Ramatla et al. 2020
*spv*C	*Spv*C-F *Spv*C-R	GGGGCGGAAATACCATCTACA GCGCCCAGGCTAACACG	60	Olobatoke & Mulugeta 2015; Ramatla 2019
*hil*A	*Hil*A-F *Hil*A-R	CGGAACGTTATTTGCGCCATGCTGAGGTAG GCATGGATCCCCGCCGGCGAGATTGTG	65	Ramatla et al. 2020
misL	*MisL*-F *MisL*-R	GTCGGCGAATGCCGCGAATA GCGCTGTTAACGCTAATAGT	58	Ramatla et al. 2020
orfL	*OrfL*-F *OrfL*-R	GGAGTATCGATAAAGATGTT GCGCGTAACGTCAGAATCAA	58	Ramatla et al. 2020
spiC	*SpiC*-F *SpiC*-R	CCTGGATAATGACTATTGAT AGTTTATGGTGATTGCGTAT	55	Ramatla et al. 2020; Zishiri et al. 2016

Table III: Primer sequences, annealing temperatures and predicted sizes of amplicons for *E. coli*

Target gene	Primers	Primer name and sequence	Annealing temperature (°C)	Product size (bp)	References
aggR	AggRks AggRkas2-	GTATACACAAAAGAAGGAAGC ACAGAATCGTCAGCATCAGC	48	254	Ndlovu et al. 2015
stx	VTcom-u VTcom-d	GAGCGAAATAATTTATATGTG TGATGATGGCAATTCAGTAT	58	518	Kumar et al. 2004
eae	SK1 SK2	CCCGAATTCGGCACAAGCATAAGC CCCGGATCCGTCTCGCCAGTATTCG	48	881	Ndlovu et al. 2015
uidA	*uidA* F *uidA* R	F- CTGGTATCAGCGCGAAGTCT R- AGCGGGTAGATATCACACTC	61	556	Anbazhagan et al. 2011

Detection of *E. coli*

The PCR targeted the amplification of the *uidA* gene encoding for adhesin of *E. coli*, *aggR* gene encoding for antiaggregative protein (*app*) gene of Enteroaggregative *E. coli, stx* (Shiga toxins) encoding for lambdoid bacteriophages and *eaeA* gene encoding for enteropathogenic *E. coli* intimin. The primers and product sizes and annealing temperatures are shown in Table III.

Statistical analysis

Chi-square was used to test for significance of infection rate between male and female rats as well as between different rat species, whereas ANOVA was used to test for variance in observations made across the different sampling sites.

Results

Rodent identification

The PCR amplification and sequencing of the *COI* gene revealed that 55 (36%) and 99 (64%) of 154 rodent samples were *R. tanezumi* and *R. rattus*, respectively. Detailed information is provided in our previous publication (Ramatla et al. 2019).

Biochemical results

One hundred and fifty-four captured rat samples were investigated for the presence of zoonotic bacterial pathogens that they were possibly carrying, of which 3.3%, 20.7% and 12.3% were harbouring *Shigella* spp., *E. coli* and *Salmonella* spp., respectively as shown in Table IV. All chemical tests for *Salmonella* spp., *Shigella* spp. and *E. coli* isolates are shown in Table IV. A total of 33 presumptive isolates were suspected to be *Shigella* spp., 120 samples were presumptive *Salmonella* isolates and 162 were *E. coli* isolates.

Molecular results

Out of five farms where samples were collected, *Shigella* spp. was not detected in two farms as shown in Table V. Based on biochemical tests, 33 presumptively positive *Shigella* isolates were identified. All 33 isolates were subjected to PCR of which 14 isolates were harbouring *ipa*H gene (613 bp) for *Shigella* spp., eight were positive for *Sson* (430bp), and five were positive for *chp* (248bp) genes for *S. sonnei* and *S. boydii*, respectively. One *ipaH*-positive isolate was negative for *S. sonnei*, *S. boydii* and *S. flexneri*, which we suspect might be *S. dysenteriae*.

The *inv*A positive isolates (n = 68) were thus confirmed as *Salmonella* spp. All the other virulence genes detected are shown in Table VI. However, the genes: *fli*C, *fli*B, and *Ppb*23 were not amplified. Among the 162 presumptive *E. coli* isolates tested, a total of 147 showed amplification of the *uidA E. coli* housekeeping gene (Table V). After running the gel on electrophoresis for 65 minutes, the desired 556 bp fragments were obtained. Therefore, all 147 samples were confirmed as *E. coli* isolates. The *aggR* (42%), and *eae* (14%) genes were detected while none of the samples had the *stx* gene.

Table IV: The distribution of pathogens among *Rattus* species

***Rattus* spp.**	**No of rodents**	***Shigella***	***Salmonella***	***E. coli***
R. rattus	99	3 (3.03%)	12 (63.2%)	20 (20.2%)
R. tanezumi	55	–	7 (36.8%)	12 (21.8%)
Total	**154**	**3 (3.03%)**	**19 (12.3%)**	**32 (20.7%)**

Table V: Number of isolates from sampled farms

Farm	**No of rats**	**Confirmed *Shigella* isolates**	**Confirmed *Salmonella* isolates**	**Confirmed *E. coli* isolates**
A	25	3	35	28
B	3	–	1	12
C	21	4	12	33
D	17	–	4	6
E	68	5	9	46
F	20	2	7	22
Total	**154**	**14**	**68**	**147**

Table VI: The prevalence of the pathogens based on biochemical tests and DNA results and the proportions of detected genes

Pathogen	No of rats tested	PCR-positive rats	Conventional biochemical identification	Confirmatory gene	Proportion of other genes
Shigella spp.	154	3.3%	33	*ipa*H 14	*Sson* (57%), *chp* (36%)
Salmonella spp.	154	29.9%	120	invA 68	*spy* (38%), *sdfI* (18%), *spv*C (20%), *hilA* (41%), *mis*L (63%), *orf*L (46%), *spi*C (56%)
E. coli	154	20.7%	162	*uid*A 147	*aggR* (42%), *eae* (14%)

Discussion

Rodents can act as reservoirs of several pathogens, and controlling them effectively leads to control of these pathogens and the diseases they cause. They primarily transmit pathogens from the farm environment through their droppings and urine to food animals and subsequently to human beings. The poultry industry in South Africa does not yet fully appreciate the significant role played by rodents in the spread of *E. coli*, *Shigella* spp. and *Salmonella* spp. contamination in poultry farms and ultimately into the food chain. These pathogens are very important causes of serious diseases, colibacillosis, shigellosis and salmonellosis in human beings (Hong et al. 2018; Zhang et al. 2018). The current study, therefore, puts into perspective the important role that these rodents may play in possible circulation of these pathogens to poultry and ultimately humans.

In this study, the *ipaH* gene of *Shigella* spp. was detected from 3.3% of screened faecal rat samples. This gene, encoding the *IpaH* protein family (Lin et al. 2010), is found in four species of *Shigella* (*S. dysenteriae, S. sonnei*, *S. boydii,* and *S. flexneri*) (Lin et al. 2010; Mokhtari et al. 2012) and is therefore used as an indicator to detect the occurrence of *Shigella* spp. (Alizadeh-Hesar et al. 2014). Other studies have also found *S. sonnei* as the predominant species (Ranjbar et al. 2008; Tajbakhsh et al. 2012), compared to *S. dysenterae* which was rarely detected (Nave et al. 2016). It has also been reported that *S. sonnei* is the most isolated serotype in chickens (Zhang et al. 2013) and in human beings (Duran et al. 2013), thus supporting our findings in this case. *S. sonnei* invades human intestines, and then spreads, which is the critical process of its mechanism for its virulence. It has been found to be one of the most common species causing shigellosis (Ranjbar et al. 2008; Tajbakhsh et al. 2012). *S. boydii* was also detected in this study. These findings are in line with previous studies whereby *S. boydii* was detected (Ranjbar et al. 2014; Ud-Din et al. 2013). These findings, therefore, show that rats are a big public health risk factor for shigellosis.

The expression of invasion factor A (*inv*A) gene has been confirmed in *Salmonella* spp. and is used for *Salmonella* spp. identification (Akinola et al. 2019; Fekry et al. 2018; Li et al. 2018; Refai et al. 2017). All the isolates which were *inv*A positive ($n = 68$) in the current study were thus confirmed as *Salmonella* spp. The *inv*A gene enables and shows the capacity of *Salmonella* spp. to infiltrate and cause gastroenteritis (Ekwanzala et al. 2017; Li et al. 2018) and is thus a marker of the pathogen's virulence. In our study, a proportion of 12.3% of *Salmonella* spp. was identified in rats, which is substantially lower than that which has been reported in other countries such as the USA with 16.2% (Henzler & Opitz 1992), Nigeria with 18.0% (Wakawa et al. 2015), as well as Japan with 31.8–41.2% (Lapuz et al. 2008). However, in this study the detection rate of *Salmonella* is higher than that found on poultry farms in other countries, including the UK with 10.0% (Hilton et al. 2002) and 2% in Trinidad and Tobago (Nkogwe et al. 2011). The proportional discrepancies could be owing to the analytical methodologies utilised, the number of samples analysed, and the various geographical/regional circumstances.

In 68 *inv*A harbouring *Salmonella* isolates, the *spy*C, *hil*A, *mis*L, *orf*L, *sdfI* and *spy* virulence genes were also detected. The *spv*C gene, which is translocated into the host cell cytoplasm by the SPI-2 TTSS (type three secretion system), was found in 21% of the isolates (Browne et al. 2008). The *spv*C gene is necessary for host cell survival and is important for systemic infections (Chaudhary et al. 2015). The *hil*A gene was carried by eight isolates (11%). This gene is significant in *Salmonella* pathogenesis because it is required for bacterial colonisation of the host intestine's extracellular luminal compartment (Peixoto et al. 2017). About 65% of the *Salmonella* isolates were carrying *mis*L gene and the gene increases pathogenicity by allowing the infection to survive inside macrophages (Hughes et al. 2008). About 47% of the isolates were carrying the *orf*L gene which helps with adhesion and survival in macrophages, and it also possesses a secretion mechanism that regulates toxin secretion (Odjadjare & Olaniran 2015), hence promoting the pathogenicity of the *Salmonella* isolate engaged. About 12 (18%) of the isolates had the *sdf*I, for the detection of *S.* Enteritidis (Mohd Afendy & Son 2015), whereas the *spy* gene was found to be present in 26 (38%), and many researchers have used it to detect *S.* Typhimurium (De Freitas et al. 2010). The *S.* Enteritidis and *S.* Typhimurium strains have been identified as causes of human diseases (Collard et al. 2008) and also infecting some animals (Rodríguez et al. 2018). Therefore, this study confirms the presence of these *Salmonella* serovars from rats and further shows that these strains are virulent.

Most *E. coli* strains are known to be commensal bacteria in all warm-blooded animals' gastrointestinal tracts (Karmali et al. 2010; Madoshi et al. 2016). The *aggR* and *eae* genes were detected in this study, while the *stx* gene was not detected from all the rat faecal samples. The findings of this study concur with previous results obtained in Tunisia (Salem et al. 2011) and in South Africa (Ndlovu et al. 2015). However, the detection of *aggR, stx* and *eae* genes among the *E. coli* isolates is of great concern. The *eae* gene, on the other hand, is found in the enteropathogenic (EPEC) *E. coli* strains and used as a marker for LEE-positive STEC strains (Alonso et al. 2017; Phillips & Frankel 2000) and is responsible for attacking and destroying the intestinal epithelial cell of the host (Malik et al. 2017). Shiga toxin (Stx) is the virulent factor in Shiga toxin-producing *E. coli* (STEC) which causes human gastrointestinal diseases (Phillips & Frankel 2000). The *aggR* is

described as a transcriptional regulator of enteroaggregative *E. coli* (EAEC). Enteroaggregative *E. coli* causes diarrhoea in humans (Morin et al. 2013). The presence of these *E. coli* strains in rats that are capable of contaminating both feed and poultry products is an important public health concern.

Conclusion

The detection of *Shigella, Salmonella* and *E. coli* bacteria in the faecal samples highlights the importance of these rodents in the spread of these pathogens among rats, chickens, and finally human beings. The fact that the strains detected also had the genes that signify virulence and are similar to species known to cause disease in humans and animals is of great significance. The rats provide the chance for environment–rat–chicken interaction during ingestion of *Shigella* spp.*, Salmonella* spp. and *E. coli*-contaminated rodent faecal droppings by poultry, thus increasing the risk of circulation and re-introduction of the pathogens even after the flock has been evacuated and the environment cleansed and disinfected. Consequently, the risks posed by the presence of rodents (*Rattus* spp.) in the transmission cycle in the human environment and in chicken farms should not be overlooked. To limit the risk of *Shigella*, *Salmonella*, and *E. coli* transmission to chickens and, ultimately, humans, it is critical to manage rats on poultry farms. Control and prevention of these pathogens within these poultry farming settings requires a "One Health" approach.

Ethical approval

Prior to the commencement of the study, the research proposal was approved based on Animal Research Ethics Committee (NWU-00274-18-A5) guidelines by North-West University Research Ethics Regulatory Committee (NWU-RERC).

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