Occurrence, distribution and virulence factors of clinically important *Acinetobacter* species recovered from selected freshwater resources in the Eastern Cape Province, South Africa

Mary Ayobami Adewoyin (✉ marymartin055@gmail.com)

University of Fort Hare <https://orcid.org/0000-0001-7489-7402>

Anthony Ifeanyi Okoh

University of Fort Hare

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Abstract

Background : Several *Acinetobacter* species live in different ecosystems such as soil, freshwater, wastewater, and solid wastes. In this study, we assessed the occurrence of *A. baumannii* and *A. nosocomialis*, which are the major two clinically important species of the genus *Acinetobacter*, in three freshwater resources (Great Fish, Keiskemba, and Tyhume rivers) in the Eastern Cape Province, South Africa over a one year sampling regime (April 2017 - March 2018). Presumptive *Acinetobacter* species were subjected to molecular identification by using *Acinetobacter* genus-specific primers targeting the *recA* gene. The confirmed *Acinetobacter* species were further delineated into *A. baumannii* and *A. nosocomialis* using species-specific primer sets. Similarly, virulence genes, namely; *afa/draBC*, *epsA*, *fimH*, *OmpA*, *PAI*, *sfa/focDE*, and *traT* in the two *Acinetobacter* species were also determined using molecular techniques.

Result : A total of 1107 presumptive *Acinetobacter* isolates were recovered from the freshwater resources of which 844 was confirmed positive for the *Acinetobacter* genus. Of the 844 *Acinetobacter* isolates, 285 (77%), 219 (70.9%) and 340 (79%) were recovered from Great Fish, Keiskemba and Tyhume rivers respectively. Our finding revealed that 410 (48.58%) and 23 (2.7%) of the isolates were confirmed to be *A. baumannii* and *A. nosocomialis*, respectively. The presence of these clinically-important *Acinetobacter* species in the freshwater studied suggests possible contamination of the selected rivers and also that *A. baumannii* and *A. nosocomialis* can thrive in aquatic environments. Besides, 308 (75.12%) *A. baumannii* and 3 (13.04%) *A. nosocomialis* isolates exhibited one or more virulence genes out of the seven tested, whereas 102 (24.88%) and 20 (86.95%) of the *A. baumannii* and *A. nosocomialis* isolates did not harbour any virulence gene. Additionally, *OmpA* was the most prevalent ($p < 0.05$) virulence gene in *A. baumannii* with 69 (45.10%), 52 (50.98%) and 77 (49.68%) isolates in Great Fish, Keiskemba and Tyhume rivers respectively.

Conclusion : The occurrence of these pathogens in rivers which are consumed by humans and livestock, as well as being used for irrigation system constitutes a risk to public health. **Keywords:** Freshwater resources, Molecular characterisation, *Acinetobacter* species, virulence genes.

Background

The majorities of *Acinetobacter* species are free-living and are ubiquitous in nature, but the clinically-important species, most especially the *Acinetobacter calcoaceticus-baumannii* (ACB) complex, are frequently isolated from the hospital environs. Among members of this complex, *A. baumannii*, *A. nosocomialis*, and *A. pittii* are well-reported causative agents of *Acinetobacter*-related infections in hospitals around the globe [1, 2]. The most virulent *Acinetobacter* species, *A. baumannii*, is known to cause disease outbreaks in intensive care units (ICUs). Owing to the importance of this disease-causing species in the clinical settings, some scientists have reported that it is a nosocomial pathogen and its occurrence in other environments is likely not possible [2]. However, in recent discoveries, *A. baumannii* has been reported to thrive in freshwater, soil, healthy human skin just like other non-pathogenic species in the Genus [3, 4]. The occurrence of *A. baumannii* and other pathogenic species of the genus *Acinetobacter* in other

environment apart from the hospital could be associated with the indiscriminate disposal of wastewater and materials from the hospital into the environment [5].

The isolation of *A. baumannii* from hospitalized patients is often associated with serious ailment, with corresponding mortality rate of approximately 30% [6, 7]. Additionally, *A. baumannii* has been implicated in a range of diseases which includes; *Acinetobacter* pneumonia-bronchiolitis and tracheobronchitis [8, 9], bloodstream infection [2, 10], wound infection [11], urinary tract infection [2, 11] and meningitis [8, 12]. Similarly, *A. nosocomialis* was responsible for bacteremia [13, 14], pneumonia [15], and induction of epithelial cell death and host inflammatory responses [16].

The pathogenicity of *Acinetobacter* species like other microorganisms is strongly associated with the virulence factors they harbour. These factors include porins (OmpA), capsular polysaccharides, lipopolysaccharides (LPS), phospholipase, outer membrane vesicles (OMVs), protein secretion systems, metal acquisition system etc. [8, 17, 18, 19]. The *OmpA* is a protein located on the outer cell membrane of the bacteria, which is responsible for the selective permeation of materials in and out of the cell. The *OmpA* also binds to the host epithelial cells in order to gain entry into the cell cytoplasmic environment. As such, it causes cell death (apoptosis) by releasing the cytochrome c. *OmpA* was also noted as one of the factors with which *A. nosocomialis* initiates its pathogenesis [20]. Pathogenic Gram-negative bacteria are known to secrete outer membrane vesicles (OMVs) [17, 21, 22], for interaction between the bacterial pathogens and the host cells [23]. Kim and others [20] showed that *A. nosocomialis* uses its outer membrane vesicles (OMVs) for secretion of cytotoxic factors with which it elicits an immune response from host epithelial cell. Phospholipase, like other factors, also contributes to the virulence of the pathogenic *Acinetobacter* species, by hydrolysing phospholipid bilayer of the host cell membrane in order to destabilize the entire cell [17, 24].

As a result, an investigation of the occurrence and distribution of these clinically-important *Acinetobacter* species in other environments, other than hospital, is very necessary for the understanding of what nature and diversity truly represent [25, 26]. As such, the time has come to redefine the environmental coverage by these organisms, beyond a clinical setting. Therefore, the focus of this study was to assess the occurrence, distribution and the virulence factors of clinically-important *Acinetobacter* species such as *A. baumannii* and *A. nosocomialis* in the selected freshwater resources in the Eastern Cape, South Africa.

Results

Isolation and distribution of presumptive *Acinetobacter* species

A total of 1107 presumptive *Acinetobacter* species was recovered from the three rivers studied, of which 370, 309 and 428 presumptive isolates belonged to Great Fish, Keiskemma and Tyhume rivers respectively as summarized in Table 1.

PCR amplification of the *recA* gene

To further validate the occurrence and distribution of the bacteria in the genus *Acinetobacter* in this study, the identification was achieved by using the PCR-based assay to detect internal *recA* genes that are specific

to all *Acinetobacter* species. Figure S1 presents the PCR product of the gel electrophoresis and staining for the amplification of the 425 bp fragment, which correlates to *recA* gene. Out of the 1107 presumptive isolates, 844 were confirmed to belong to the genus *Acinetobacter* of which 285 (77%), 219 (70.9%) and 340 (79%) were recovered from Great fish, Keiskemma and Tyhume rivers respectively. The confirmed isolates belonging to the genus *Acinetobacter* is summarized and presented in Table 1.

Delineation of Genus *Acinetobacter* into species

Figures S2A and S2B shows the PCR products of the gel electrophoresis and staining for the delineation of the genus *Acinetobacter* into *A. baumannii* and *A. nosocomialis* accordingly, while *A. pittii* was not detected.

The occurrence of *A. baumannii* was 153 (53.68%) in Great Fish river, 102 (46.58%) in Keiskamma river, and 155 (45.59%) in Tyhume river, while 16 (5.61%), 3 (1.37%) and 4 (1.18 %) represent *A. nosocomialis* from the respective rivers. The presence of *A. baumannii* in the three rivers studied was significantly higher ($p < 0.05$) than the *A. nosocomialis* isolates. The delineation into *A. baumannii* and *A. nosocomialis* are summarized and presented in Table 1.

Detection of Virulence Genes

The gel electrophoresis of the virulence genes; *afa/draBC*, *epsA*, *fimH*, *OmpA*, PAI, *sfa/focDE*, and *traT*, are presented in Figure S3. The virulence genes harboured by *A. baumannii* and *A. nosocomialis* in this study is presented in Figure 2 and summarized in Table 2. Additionally, the virulence gene(s) associated with each of the *Acinetobacter* species as well as the river source from which they were isolated is described. The statistical significance of the prevalence virulence genes with respect to rivers was also emphasized.

Three hundred and eight (75%) of the 410 *A. baumannii* and 3 (13%) of the 23 *A. nosocomialis* isolated from the rivers were observed to exhibit one or more virulence genes, out of the seven tested. Respectively, 102 (24.88%) and 20 (86.95%) of the *A. baumannii* and *A. nosocomialis* isolates from the three rivers did not harbour any of the virulence genes tested in this study. However, there was a significant difference ($p < 0.05$) in the prevalence of virulence genes harboured by *A. baumannii* compared to *A. nosocomialis*, though the population of the former was significantly higher than the latter in all cases.

The prevalence of virulence gene *afa/draBC* was significantly higher ($p < 0.05$) in the isolates from Great Fish river in comparison to Keiskamma and Tyhume rivers. However, there was a significant difference ($p < 0.05$) in prevalence between the *afa/draBC* genes harboured by the bacteria recovered from the Tyhume river when compared to those from Keiskamma river. Besides, the prevalence of *traT* gene in *Acinetobacter* species from Great Fish and Tyhume rivers showed no statistical difference ($p < 0.05$), but both were significantly higher ($p < 0.05$) than those detected in isolates from Keiskamma river. Similarly, the prevalence of *fimH* gene detected in isolates recovered from Great Fish river was significantly higher ($p < 0.05$) than those detected in isolates from both Keiskamma and Tyhume river. Nonetheless, the proportion of isolates harbouring the gene in Keiskamma was significantly ($p < 0.05$) lower than those from Tyhume river. The prevalence of the PAI virulence gene was not significantly ($p < 0.05$) different in all the rivers studied. In Great

Fish and Keiskamma rivers, there was no significant difference ($p < 0.05$) in the prevalence of *sfa/focDE* in isolates recovered from the rivers, but the proportion of *Acinetobacter* species in Tyhume harbouring the gene was significantly higher ($p < 0.05$) than in those from both Great Fish and Keiskamma rivers. The number of *Acinetobacter* species harbouring *epsA* virulence gene in the Great Fish river was significantly higher ($p < 0.05$) than those recovered from Keiskamma river, but significantly lower ($p < 0.05$) than in *Acinetobacter* species recovered from Tyhume river. The prevalence of the *OmpA* virulence gene was significantly higher ($p < 0.05$) in *A. baumannii* isolates than all other virulence genes in this study. Besides, *OmpA* was the most prevalent virulence gene in the rivers, which represented 69 (45.10%), 52 (50.98%) and 77 (49.68%) *A. baumannii* isolates in Great Fish, Keiskamma and Tyhume rivers respectively. The number of *Acinetobacter* species harbouring *OmpA* gene in Great fish river were significantly higher ($p < 0.05$) than isolates from Keiskamma river, whereas Tyhume river maintained the highest level of statistical significance ($p < 0.05$) of the total *Acinetobacter* species exhibiting the virulence gene.

Summarily, *OmpA* was the most prevalent virulence gene detected in *Acinetobacter* species in all the three rivers studied followed by *fimH* and *epsA* genes, whereas PAI and *Sfa/focDE* genes were the least exhibited respectively. Furthermore, the number of *Acinetobacter* species harbouring the highest percentage of virulence genes was isolated from the Tyhume River followed by the Great Fish River, while Keiskamma River was the least.

Discussion

Bacteria in the genus *Acinetobacter* have been known to colonize a wide array of ecological systems of which water, soil, sludge, wastewater, plants' root, and animals have been prominent. In this study, water samples were collected at five different sites from three selected rivers in the Eastern Cape Province, South Africa for the assessment of the of *Acinetobacter* species. Several presumptive species belonging to the genus *Acinetobacter* were recovered from the water samples. Similar study was carried out by [27] on the microbial community in the urban riverine environment in Croatia, where 57 of the isolates belong to the genus *Acinetobacter*. Likewise, Krizova et al. [28], noted a widespread of *A. bohemicus* in the water environment in the Czech Republic. *A. baumannii* was characterized from a surface water resources in South Nation River (SNR) drainage basin in Eastern Ontario, Canada, in 2013 [26]. Besides, the composition of the bacterial community in a freshwater aquaculture environment was investigated in China by [29]. The study reported that the relative abundance of *Acinetobacter* species was 0.5% of the total bacterial community.

All isolates that were positive for the *recA* (425 bp) (Figure S1) were taken as belonging to the *Acinetobacter* genus, according to [30]. A PCR amplification assay based on *recA* gene-specific primer, identified 844 *Acinetobacter* species of the 1107 presumptive isolates, while *gyrB* gene species-specific primers further delineated them into species namely, *A. baumannii* (410) and *A. nosocomialis* (23). According to [30], the method used in this study was 98.2% specific and 92.4% sensitive for Comparably, [31] demonstrated a PCR assay targeting *recA* gene *A. baumannii*, *A. nosocomialis*, and *A. pittii* are known nosocomial pathogens, which could cause multiple antibiotic resistant infections [2, 30, 32] in immune-compromised patients [2]. Park and others [33] investigated the presence of *Acinetobacter* species in the bloodstream of

patients with blood infection in a tertiary-care hospital in Korea between August 2003 and February 2010. Their findings showed that *A. baumannii* and *A. nosocomialis* were prevalent in the samples collected. Similarly, [34], recovered 160 *A. baumannii* isolates from sputum, blood, pus and fluid aspirates of patients in three Hospitals in Vietnam within a period of two years (between 2012 and 2014). These findings showed that the nosocomial pathogens could colonize any part of the human body to cause infections.

However, occurrence of *A. baumannii* and *A. nosocomialis* in an aquatic environment, is uncommon in the past few years. Nonetheless, in recent times, these nosocomial pathogens are isolated from other ecosystems [3, 4, 35]. The isolation of the pathogen from water sources might be due to contamination coming from hospital wastewater and materials.

The virulence traits of *A. baumannii* and *A. nosocomialis* have been a major research focus in recent times [17, 36]. This is due to the nature of *A. baumannii* infections as well as the role virulence genes play in the pathogenicity of the emerging waterborne pathogen. Virulence genes are the mechanisms through which *A. baumannii* initiates pathogenesis [36, 37], most especially in the clinical settings.

In this study, molecular assay for the detection of virulence factors in both pathogens was performed. Figure S2 shows the electrophoretic images of the seven virulence factors evaluated. Although, *A. baumannii* had been confirmed to harbour several virulence factors (genes), seven of these genes (*afa/draBC*, *epsA*, *fimH*, *OmpA*, PAI, *sfa/focDE*, and *traT*) are reported in this study. Reports on the virulence genes in *A. baumannii* are usually associated with isolates from clinical environment, whereas such reports are rare on *A. baumannii* from freshwater resources

Thus, this study showed that *OmpA* gene was predominantly exhibited by the *A. baumannii* in all the rivers sampled, likewise *fimH* and *epsA* genes were also detected in many of the isolates, whereas *afa/draBC*, PAI, *Sfa/focDE*, and *traT* genes were detected in a few *Acinetobacter* isolates. As such, the exhibition of virulence genes varies from one isolate to the other, which was also reported among clinical isolates known for nosocomial infections [38]. The *OmpA* gene is the main outer membrane protein (OMP) located on the *A. baumannii* membrane [39, 40], which [36]. These observations corroborate the findings of this current study which reports that the virulence profiles of an individual isolate varied greatly and *OmpA* gene was mostly detected [36].

Generally, the outer membranes of Gram-negative bacteria are made up of the OMPs, lipopolysaccharides and phospholipids layer [37]. The presence of outer membrane protein A gene (*OmpA*) in the *A. baumannii* isolated from the freshwater source is of a major concern based on its role in pathogenesis [36]. Besides, studies have shown that *A. baumannii* uses *OmpA* for adhesion to the lung epithelial cell by interacting with a cell cytoskeleton such as fibronectin on the cell surface and thereby inducing pneumonia [41, 42]. It also causes cell death through caspase-3 activation [43, 44]. Similarly, *A. baumannii* could be responsible for apoptosis through the translocation of its *OmpA* into the mitochondria and the nucleus of host cells [45, 46]. The combination of the roles played by *OmpA* makes it an important virulence factor in the pathogenesis of *A. baumannii* infection. Moreover, antibiotic resistance in *A. baumannii* is also associated with *OmpA* [47, 48]. It was suggested that *OmpA* was involved in the removal of antibiotics from the

periplasmic space membrane efflux systems [48]. The survival and persistence of *A. baumannii* in the cell are enhanced by *OmpA* due to the formation of biofilms and surface motility.

Conclusions

In this study, three selected freshwater resources in the Eastern Cape Province, South Africa were evaluated for the occurrence, distribution and virulence genes fingerprints of clinically-relevant *Acinetobacter* species namely; *A. baumannii* and *A. nosocomialis*. This study suggests that these pathogenic *Acinetobacter* species could also inhabit aquatic environment. As a result, aquatic environment is an important reservoir for pathogenic *Acinetobacter* species as detected in this study. Besides, the *OmpA* is a major virulence factor associated with *A. baumannii* and *A. nosocomialis* in this study. As such, identification of these opportunistic and virulent waterborne pathogens in the freshwater resources requires public awareness and recognition as important to public health risks.

Methods

Description of study areas

Collection of water samples was carried out from three rivers namely; Great Fish, Keiskamma and Tyhume, in the Eastern Cape Province, South Africa between April 2017 and March 2018. The Great Fish River is located in Chris Hani District Municipality in the Eastern Cape Province and it is one of the major rivers used for irrigation and livestock farming in the area. This river is prone to agricultural and municipal runoffs and also serves as the receiving stream of effluents from many wastewater treatment plants (WWTPs), especially those situated in urban communities such as Craddock. Keiskamma and Tyhume rivers are located in the Amathole District Municipality in the Eastern Cape Province and are exposed to different anthropogenic activities from the rural and urban communities along the river courses, such as livestock drinking and irrigation farming. In addition, these rivers receive effluents from wastewater treatment plants (WWTPs) situated close to their banks. Different sampling points on these rivers were selected based on where humans and animals come into direct contact with them, for example, points where they are used for fishing, drinking and swimming purposes, downstream of the WWTPs, points where irrigation water is released to the water bodies and proximity to hospital facilities.

Sampling

Water samples were collected at five different sites (S1, S2, S3, S4, and S5) from Great Fish, Keiskamma and Tyhume rivers respectively, for a period of one year, which covers the four seasonal patterns in South Africa (autumn, winter, spring, and summer). Water samples were collected aseptically in sterile 1L glass bottles from different sampling points by midstream-dipping of sample bottles at 25–30 cm down the water column, with the mouth tilting against the flow of the river. All water samples were labelled properly and safely taken to the laboratory (in an ice chest) where they were processed within 6 h of collection [27]. Aliquots of water samples were used for isolation of *Acinetobacter* species based on standard microbiological procedures [49]

Isolation and purification of presumptive *Acinetobacter* species

The isolation of the presumptive density of *Acinetobacter* species in the water samples was determined by membrane filtration technique [50]. Cellulose membrane of pore size 0.45 μm was used to filter three volumes of 100 mL of the water samples under vacuum [50]. These membranes were aseptically placed on plates with *Acinetobacter* species selective medium-CHROMagar *Acinetobacter* base plus selective supplement (CHROMagar, Paris, France) which was prepared according to the manufacturer's instruction. Each sample plate was subjected to incubation at 37°C for 24 h after inoculation. Each sample was analysed in triplicate. All bacterial colonies with red colouration on the CHROMagar plates were counted as presumptive *Acinetobacter* species and were expressed as CFU/100ml. All isolates were sub-cultured on nutrient agar using a streak plate method (Oxoid, UK) and purified for further species identification. Fifty percent (50%) glycerol stocks of the pure culture was prepared and stored at -80 °C.

Molecular identification of *Acinetobacter* species by PCR assays

Extraction of genomic DNA: Presumptive *Acinetobacter* spp. in glycerol stocks was first being resuscitated on tryptic soy broth and incubated for 18 to 24h at 37 °C. DNA extraction from the bacterial isolates was carried out using the direct boiling method according to [51]. The broth culture was centrifuged at 15000 *rpm* for 5 min using a Mini Spin Microcentrifuge (Lasec, RSA), then the supernatant was dispensed out and the pellet rinsed with sterile normal saline. The pellet was re-suspended in sterile distilled water and boiled in a heating block for 10 minutes using an AccuBlock (Digital dry bath, Labnet).

Amplification of unique *Acinetobacter* species DNA

Polymerase chain reaction (PCR) assay was used for the amplification of the *Acinetobacter* species *recA* gene as previously described [30]. The forward and reverse primers used were P-rA1 (5'-CCTGAATCTTCTGGTAAAC-3') and P-rA2 (5'-GTTTCTGGGCTGCCAAACATTAC-3') respectively. Briefly, an aliquot of 25 μl containing Taq PCR (12.5 μl) Master Mix (Qiagen, Hilden, Germany), each of the primers (1 μl) (Inqaba, SA), nuclease-free water (6.5 μl) and DNA template (5 μl) was used for the PCR amplification assay. The condition for the amplification included initial denaturation step (94 °C, 5 min), followed by 35 cycles (92 °C, 40 s), annealing (58 °C, 40 s), and the final extension step (72°C, 10 min) was performed using a thermocycler (Bio-Rad Thermal cycler, USA). Five microlitres (5 μl) of the amplicon was subjected to gel (1.5% agarose) electrophoresis at 100 Volts for 45 min in Tris Boric EDTA buffer (pH 8.0) (0.089 M Tris, 0.089 M boric acid, and 0.002 M EDTA). Ethidium bromide (5 μl of 0.5 mg/ml) (Sigma-Aldrich, USA) was used for gel staining and DNA ladder (100 bp) (Thermo Scientific, (EU) Lithuania) was added into the gels as a standard. Finally, DNA bands were visualized under an ultraviolet transilluminator (Alliance 4.7, France).

Delineation of Genus *Acinetobacter* into species

The confirmed *Acinetobacter* isolates were delineated into species accordingly [30, 52, 53] using a PCR assay. Firstly, optimization of conditions for *A. baumannii*, *A. nosocomialis* and *A. pittii* was carried out using species-specific primers (Table S1 under supplementary Section), while reference strains DSM-

102929, DSM-102856, and DSM-9341 (DSMZ, Germany) were used as positive controls for *A. baumannii*, *A. nosocomialis* and *A. pittii* respectively. The PCR amplification was performed as stated in the previous section.

Detection of virulence genes

Polymerase chain reactions were also carried out for the identification of some *Acinetobacter* virulence genes including *afa/draBC*, *epsA*, *fimH*, *OmpA*, PAI, *sfa/focDE*, and *traT* genes, which have been previously found in clinical samples [54, 55, 56]. The standard strain of *A. baumannii* DSM-30007 (DSMZ, Germany) was used as positive control. There was no positive control available for *A. nosocomialis*. The PCR assay and electrophoresis were conducted as earlier described.

Statistical Analysis

All statistical analyses were performed using the Statistica software v13.4.0.14 (64-bits). A simple factorial ANOVA was performed for the comparison of normally distributed data. The *p*-values of less than 0.05 were considered statistically significant for all the statistical tests performed.

Abbreviations

ACB: *Acinetobacter calcoaceticus*- *baumannii* complex;

ANOVA: Analysis of Variance;

APHA: American Public Health Association;

CFU: Colony Forming Unit;

CHROMagar: Chromogenic agar;

DNA: Deoxyribonucleic Acid;

EDTA: Ethylenediamine tetraacetic acid;

EspA: Exopolysaccharide A;

EU: European Union;

ICU: Intensive care unit;

L: Ladder;

LPS: Lipopolysaccharides;

N: Negative control;

ND: Not determined;

NRF: National Research Foundation;

ompA: Outer membrane protein A precursor;

OMVs: Outer membrane vesicles;

P: Positive control;

PAI: Pathogenicity Island;

PCR: Polymerase Chain Reaction;

RSA: Republic of South Africa;

SAMRC: South African Medical Research Council;

TWAS: The World Academy of Science;

UFH: University of Fort Hare;

UK: United Kingdom;

USA: United States of America;

WWTPs: Wastewater treatment plants.

Declarations

Ethics approval and consent to participate

Collection of freshwater samples for research purposes in this region does not require governmental permission since freshwater resources investigated are within the University jurisdiction. Also, the need for ethical approval was waived by the Research Ethics Committee, University of Fort Hare, because samples analysed in this study do not require human or animal subjects.

Consent for publication

Not applicable

Availability of data and materials

The data used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MAA conceived and designed the experiment, collected the water samples, isolated the *Acinetobacter* spp., performed the experiments, data and statistical analyses, and writing of the manuscripts.

AIO supervised the research, provided financial support, and reviewed the manuscript.

All authors read and approved the final manuscript.

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Authors' information

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Tables

Table 1: Summary of the relative abundance of the genus *Acinetobacter* and two clinically important species in the freshwater studied

Rivers sampled	Presumptive isolates	<i>Acinetobacter</i> genus	Delineated <i>Acinetobacter</i> species <i>A. baumannii</i> <i>A. nosocomialis</i>		Other species (ND)
Great Fish	370	285(77.01%)	153(53.68%)	16(5.61%)	116(40.70%)
Keiskamma	309	219(70.87%)	102(46.58%)	3(1.37%)	114(52.06%)
Tyhume	428	340(79.44%)	155(45.59%)	4(1.18%)	181(53.24%)
Total	1107	844(76.24%)	410(48.58%)	23(2.73%)	411(48.70%)

ND= not determined

Table 2: Virulence gene factors of *A.baumannii* and *A. nosocomialis* isolates in addition to which river they belong to.

Virulence gene	Sample sites					
	Great Fish		Kieskamma		Tyhume	
	<i>A. baumannii</i>	<i>A.</i>	<i>A.</i>	<i>A.</i>	<i>A.</i>	<i>A.</i>
	(n=153)	<i>nosocomialis</i>	<i>baumanni</i>	<i>nosocomialis</i>	<i>baumanni</i>	<i>nosocomialis</i>
		<i>s</i>	<i>i</i>	<i>s</i>	<i>i</i>	<i>s</i>
		(n=16)	(n=102)	(n=3)	(n=155)	(n=4)
<i>Afa/draBC</i>	16(10.46%)	-	4 (3.92%)	-	8 (5.81)	1 (25%)
<i>espA</i>	36 (23.53%)	-	15 (14.71%)	-	44 (28.39%)	1 (25%)
<i>fimH</i>	44 (28.76%)	-	26 (25.49%)	1(33.33%)	30 (19.36%)	1 (25%)
<i>OmpA</i>	69 (45.10%)	-	52 (50.98%)	-	77 (49.68%)	-
PAI	2 (1.31%)	1(6.26%)	3 (2.94%)	-	-	-
<i>Sfa/focDE</i>	3 (1.96%)	-	4 (3.92%)	-	7 (4.52%)	-
<i>traT</i>	19 (12.42%)	-	4 (3.92%)	-	22 14.19%)	-

Figures

Figure 1

Isolates of *A. baumannii* and *A. nosocomialis* recovered from each of the sampling sites in Great Fish (GF), Keiskamma (KE) and Tyhume (TY) Rivers. The number of isolates presented in this here represent a pool of the five sampling sites.

Figure 2

Virulence genes in both *Acinetobacter* species recovered from each of the rivers.

Figure 3

PCR analyses resolved by gel electrophoresis showing confirmed *A. nosocomialis* targeting the *gyrB* gene at 294 bp. L= DNA Ladder (100bp); P= Positive control (*A. nosocomialis*, DSM 102856); Lanes 1 to 10 = Selected *A. nosocomialis* samples.

Figure 4

PCR analyses resolved by gel electrophoresis showing confirmed virulence genes *OmpA*, *PAI*, *fimH*, *sfa/focDE*, *espA* and *traT* at 531, 930, 506, 410, 451 and 290 base pairs respectively. L= DNA Ladder (100bp); Lane 1 to 9 (overall) = Selected *Acinetobacter* isolates; N= Negative control; P=Positive control (*A. baumannii*, DSM Number: 102929).

Figure 5

Confirmed virulence genes in the *Acinetobacter* species recovered from each of the rivers.