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

Research article

Keywords: Freshwater resources, Molecular characterisation, Acinetobacter species, virulence genes

Posted Date: September 25th, 2019

DOI: https://doi.org/10.21203/rs.2.14951/v1

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, Keiskemma, 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, Keiskemma 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. nosocomalis , 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, Keiskamma 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 gene s.

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 natural environments is likely not possible (Peleg et al., 2008). However, in recent discoveries, *A. baumannii* has been reported to thrive in other natural environment such as 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 the natural environment 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 secret outer membrane vesicles (OMVs) [17, 21, 22], for interaction between the bacterial pathogens and the host cell [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 pathogens in other environments, other than hospital, is very necessary for the understanding of what the nature and diversity of these pathogens 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* spp. was recovered from the three rivers studied, of which 370, 309 and 428 isolates belonged to Great fish, Keiskemma and Tyhume 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 1 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

The speciation of the genus *Acinetobacter* was also performed using PCR assay to amplify the *gyrB* gene specific primers of *A. baumannii* and *A. nosocomailis* at 208 bp and 294 bp fragments respectively. Figures 2 and 3 shows the PCR products of the gel electrophoresis and staining for the delineation of the genus *Acinetobacter* into *A. baumannii* and *A. nosocomailis* accordingly. From the result, 410 (48.58%) and 23 (2.73%) of the confirmed *Acinetobacter* isolates were delineated to be *A. baumannii* and *A. nosocomailis*. 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. nosocomailis* are summarized and presented in Table 1.

Detection of Virulence Genes

The schematic representations showing the gel electrophoresis of the virulence genes; *afa/draBC*, *epsA*, *fimH*, *OmpA*, PAI, *sfa/focDE*, and *traT*, are presented in Figure 4. The virulence genes haboured by the two clinically important *Acinetobacter* species in this study is presented in Figure 5 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 gene 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 haboured 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 Tyhune 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 *Acienotobacter* 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 amount of *Acinetobacter* species habouring the highest number of virulence genes was isolated from the Tyhume River followed by the Great Fish River, while Kieskamma 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 from three selected rivers in the Eastern Cape Province, South Africa for the assessment of the incidence of *Acinetobacter* species, and quite a good number of bacteria in the genus *Acinetobacter* were presumptively recovered from the water samples. In recent times, freshwater resources have been noted to be a hotspot of *Acinetobacter* species. Maravić et al. [27] carried out an assessment of the microbial community in the urban riverine environment in Croatia, where 57 of the

isolates belong to the genus *Acinetobacter*. Krizova et al. [28] also noted the widespread of *Acinetobacter bohemicus* sp. nov. in the water environment in the Czech Republic. In India, Arsenic-resistant *Acinetobacter soli, Acinetobacter venetianus, Acinetobacter junii, Acinetobacter baumannii* and *Acinetobacter calcoaceticus* were reported [29].

Besides, the composition of the bacterial community in a freshwater aquaculture environment was investigated in China by [30]. The study reported that the relative abundance of *Acinetobacter* species was 0.5% of the total bacterial community. In 2014, Zhao and others [31] isolated a set of bacteria from the zinc and arsenic polluted river in Northeastern China, where bacteria belonging to the genus *Acinetobacter* were found to be prominent. The occurrence and distribution of *Acinetobacter* spp. in freshwater resources in the study further validated the findings in other reports [29, 31].

All isolates that were positive for the *recA* (425 bp) (Figure 1) were taken as belonging to the *Acinetobacter* genus. Chen et al [32] developed a multiplex PCR amplification assay for the initial molecular identification of *Acinetobacter* species using a *recA* gene-specific primer before delineating them into species namely, *Acinetobacter baumannii, Acinetobacter nosocomialis* and *Acinetobacter pittii* by using species-specific primers. Comparably, Chiang et al. [33] demonstrated a PCR assay targeting *recA* gene for the detection of *Acinetobacter baumannii* in patients suffering from endotracheal aspirates in the intensive care unit (ICU) of Taipei Veterans General Hospital.

Eight hundred and fourty-four isolates positive for the *Acinetobacter* genus were further delineated into its species. Figure 2 and 3 represents gel images of the amplicons of the expected band size of 208 bp for *Acinetobacter baumannii* and 294 bp for *Acinetobacter nosocomialis* with respect to the *gyrB* gene. *Acinetobacter baumannii*, *Acinetobacter nosocomialis,* and *Acinetobacter pittii* are known nosocomial pathogens, which could cause multiple antibiotic resistant infections [2, 32, 34] in immune-compromised patients [2]. Park and others [35] investigated the presence of *Acinetobacter* species in the bloodstream of patients with a blood infection in a tertiary-care hospital in Korea between August 2003 and February 2010. Their findings showed that *Acinetobacter baumannii* and *Acinetobacter nosocomialis* were prevalent in the samples collected. Similarly, Anh et al. [36], 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. Sileem et al. [37], in addition to others, carried out a study on the occurrence of *Acinetobacter baumannii* in the ICU and its impact on the mortality rate.

In the present study, *Acinetobacter baumannii* and *Acinetobacter nosocomialis* were recovered from freshwater resources. Several *Acinetobacter* species have been recovered from soil, water, and

wastewater environments; however, the occurrence of *A. baumannii* and *Acinetobacter nosocomialis* in a natural environment, besides hospital settings, has been uncommon in the past few years. But in recent times, it is now very clear that such nosocomial pathogens could be isolated from other ecosystems [3, 4, 38]. Fernando et al. [26] showed evidence of the occurrence of *Acinetobacter baumannii* in the surface water resources collected from the South Nation River (SNR) drainage basin in Eastern Ontario, Canada, in 2013. Goswami et al. [29] also isolated *A. baumannii* from the river in West Bengal, India. The isolation of the pathogen from water sources might be due to contamination coming from hospital wastewater and materials.

The virulence traits of the clinically important *Acinetobacter* species such as *A. baumannii* and *A. nosocomialis* have been a major research focus in recent times [17, 39]. 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 [39, 40, 41], most especially in the clinical settings. In this study, the occurrence of these pathogens in the freshwater resources was evaluated, and the virulence factors harboured by them were also considered. Although, *A. baumannii* had been confirmed to harbour several virulence factors (genes), seven of these (*afa/draBC*, *epsA*, *fimH*, *OmpA*, PAI, *sfa/focDE*, and *traT*) are reported in this study. Most of the reports on the virulence genes harbored by *A. baumannii* are usually associated with isolates from clinical environment, whereas there are rare reports on *A. baumannii* from a natural environment harboring virulence genes. Findings of this study showed that *OmpA* gene was predominantly exhibited by the *A. baumannii* in all the rivers sampled, *fimH* and *epsA* genes were also detected in many of the isolates, whereas *afa/draBC*, PAI, *Sfa/focDE*, and *traT* 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 [42]. The *OmpA* gene is the main outer membrane protein (OMP) located on the *A. baumannii* membrane [43, 44], which readily influence the virulence of an *A. baumannii* isolate [39]. These observations corroborate the findings of this current study which report that the virulence profiles of individual isolate varied greatly and *OmpA* was mostly detected [39].

Generally, the outer membranes of Gram-negative bacteria are made up of the OMPs, lipopolysaccharides and phospholipids layer [41]. The presence of outer membrane protein A gene (*OmpA*) in the *A. baumanni* isolated from the natural environment is of a major concern based with respect to pathogenesis [39, 40, 41]. 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 [45, 46, 47, 48, 49]. It also causes cell death through caspase-3 activation [50, 51]. Similarly, *A. baumannii* could be responsible for apoptosis through the translocation of its *OmpA* into the mitochondria and the nucleus of host cells [47, 52, 53]. 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* [54, 55]. It was suggested that *OmpA* was

involved in the removal of antibiotics from the periplasmic space membrane efflux systems [55]. 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 important *Acinetobacter* species namely; *Acinetobacter baumannii* and *Acinetobacter nosocomialis*. This study suggests that these pathogenic *Acinetobacter* species could also inhabit aquatic environment. As a result, the natural environment is an important reservoir for pathogenic *Acinetobacter* species as detected in this study. Besides, the *OmpA* is the major virulence factor associated with *Acinetobacter baumannii* and *Acinetobacter 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 on 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 from Great Fish, Keiskamma and Tyhume rivers respectively, for a period of one year to cover 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 [56].

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 [57]. Cellulose membrane of pore size 0.45 μm was used to filter three volumes of 100 mL of the water samples under vacuum [57]. 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' instruction. Each sample plate was subjected to incubation at 37^{o}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* spp. 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 [58]. 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 of was used for the amplification of the *Acinetobacter* spp *recA* gene as previously described [32]. The forward and reverse primers used were P-rA1 (5'-CCTGAATCTTCTGGTAAAAC-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 ^{o}C, 5 min), followed by 35 cycles (92 ^{o}C, 40 s), annealing (58 ^{o}C, 40 s), and the final extension step (72^{o}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 strains

The confirmed *Acinetobacter* isolates were delineated into species accordingly [32] using a PCR assay. The sequences of the primer used are listed in Table 3. Here, 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 [61, 62]. The list of primers used and their annealing temperatures are shown presented in Table 4. 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 and also the

need for ethical approval was waived by the Research Ethics Committee, University of Fort Hare, since 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.

Funding

The research was funded by the National Research Foundation, South Africa/The World Academy of Science, Italy (NRF/TWAS) (grant numbers: 99767 and 116387) and the South African Medical Research Council (grant number: SAMRC/UFH/P790). The funders had no role in the study design, data collection and analysis, decision to publish, or manuscript preparation.

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.

Acknowledgements

Not applicable

Authors' information

Not applicable.

References

1. De Vos D, Jean-Paul P, Florence B, Serge J, Gilbert V, Thomas R, Elkana K, Petra B, Thierry P, Mony H, Walter H, Frank DP, Patrick S, Maia M, Pieter D, Mario V, Pierre B, Youri G, Bruno P, Tanny JR, Dijkshoorn L. Molecular Epidemiology and Clinical Impact of *Acinetobacter calcoaceticus-baumannii* complex in a Belgian Burn Wound Center. PLoS ONE. 2016; 11(5): e0156237.

2. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev. 2008; 21, 538–582.

3. Hamouda A, Findlay J, Al Hassan L, Amyes SG. Epidemiology of *Acinetobacter baumannii* of animal origin. J. Antimicrob. Agents. 2011; 38, 314–318.

4. Huys G, Bartie K, Cnockaert M, Hoang Oanh DT, Phuong NT, Somsiri T, Chinabut S, Yussoff FD, Shariff M, Giacomini M, Teale A, Swings J. Biodiversity of chloramphenicol-resistant mesophilic heterotrophs from Southeast Asian aquaculture environments. Microbiol. 2007; 158, 228–235. doi:10.1016/j.resmic.2006.12.011.

5. Hrenovic J, Durn G, Goic-Barisic I, Kovacic A. Occurrence of an Environmental *Acinetobacter baumannii* Strain Similar to a Clinical Isolate in Paleosol from Croatia. Applied and Environmental Microbiology, 2014; 80(6): 2860–2866.

6. Almasaudi SB. *Acinetobacter* as nosocomial pathogens: Epidemiology and resistance features. Saudi Journal of Biological Sciences. 2018; 25, 586–596.

7. Jung J, Park W. Acinetobacter species as model microorganisms in environmental microbiology: current state and perspectives. Appl. Microbiol. Biotechnol. 2015; 99, 2533–2548.

8. Doughari HJ, Ndakidemi PA, Human IS, Benade S. The ecology, biology and pathogenesis of Acinetobacter spp.: an overview. Microbes Environ. 2011; 26, 101–112.

9. Whitman TJ, Qasba SS, Timpone JG, Babel BS, Kasper MR, English JF, Sanders JW, Hujer KM, Hujer AM, Endimiani A. Occupational transmission of *Acinetobacter baumannii* from a United States serviceman wounded in Iraq to a health care worker. Infect. Dis. 2008; 47, 439–443.

10. Garnacho-Montero J, Amaya-Villar R, Ferrandiz-Millon C, Dıaz-Martın A, Lopez-Sanchez JM, Gutierrez-Pizarraya A. Optimum treatment strategies for carbapenem-resistant *Acinetobacter baumannii* Expert Rev. Anti. Infect. Ther. 2015; 13(6): 769-77.

11. Falagas ME, Vardakas KZ, Kapaskelis A, Triarides NA, Roussos NS. Tetracyclines for multidrug-resistant *Acinetobacter baumannii* International Journal of Antimicrobial Agents. 2015; 45, 455–460.

12. Basri R, Zueter AR, Mohamed Z, Alam MK, Norsa'adah B, Hasan SA, Hasan H, Ahmad F. Burden of bacterial meningitis: a retrospective review on laboratory parameters and factors associated with death in meningitis, Kelantan Malaysia. Nagoya J. Med. Sci. 2015; 77: 59-68.

13. Lai HH, Liou BH, Chang YY, Kuo SC, Lee YT, Chen TL, Fung CP. Risk factors and clinical outcome of sulbactam non-susceptibility in monomicrobial *Acinetobacter nosocomialis* Journal of Microbiology, Immunology and Infection. 2016; 49, 371-377.

14. Liu YM, Lee YZ, Kuo SC, Chen TL, Liu CP, Liu CE. Comparison between bacteremia caused by *Acinetobacter pittii* and *Acinetobacter nosocomialis*. Journal of Microbiology, Immunology and Infection. 2017; 50, 62–67.

15. Lee YT, Kuo SC, Yang SP, Lin YT, Chiang DH, Tseng FC, Chen TL, Fung CP. Bacteremic nosocomial pneumonia caused by *Acinetobacter baumannii* and *Acinetobacter nosocomialis*: a single or two

distinct clinical entities. European Society of Clinical Microbiology and Infectious Diseases, CMI. 2012; 19, 640–645.

16. Nho JS, Jun SH, Oh MH, Park TI, Choi CW, Kim S, Choi CH, Lee JC. *Acinetobacter nosocomialis* secretes outer membrane vesicles that induce epithelial cell death and host inflammatory responses. Microbial Pathogenesis. 2015; 81, 39-45.

17. Lee CR, Lee JH, Park M, Park KS, Bae IK, Kim YB, Cha CJ, Jeong BC, Lee SH. Biology of *Acinetobacter baumannii:* Pathogenesis, Antibiotic Resistance Mechanisms, and Prospective Treatment Options. Frontiers in Cellular Infection Microbiology. 2017; *7*(March) https://doi.org/10.3389/fcimb.2017.00055.

18. Kurcik-Trajkovska B. *Acinetobacter* – a serious enemy threatening hospitals worldwide. Macedonian Journal of Medical Science. 2009; 2, 157–162.

19. Kanafani AZ, Kanj SS. Ministry of Health, Kingdome of Saudi Arabia. 2014. http://www.uptodate.com/contents/acinetobacterinfection-treatment-and-prevention.

20. Kim SW, Oh MW, Jun SH, Jeon H, Kim S, Kim K, Lee YC, Lee JC. Outer Membrane Protein A plays a role in pathogenesis of *Acinetobacter nosocomialis*. Virulence. 2016; 7:4, 413-426, DOI: 10.1080/21505594.2016.1140298.

21. Ellis TN, Kuehn MJ. Virulence and immunomodulatory roles of bacterial outer membrane vesicles. Microbiology and Molecular Biology Review. 2010; *74*, 81–94. doi: 10.1128/MMBR.00031-09.

22. Kwon SO, Gho YS, Lee JC, Kim SI. Proteome analysis of outer membrane vesicles from a clinical *Acinetobacter baumannii* FEMS Microbiology Letter. 2009; 297, 150–156. doi: 10.1111/j.1574-6968.2009.01669.

23. Jun SH, Lee JH, Kim BR, Kim SI, Park TI, Lee JC, Lee YC. *Acinetobacter baumannii* outer membrane vesicles elicit a potent innate immune response via membrane proteins. PLoS ONE. 2013; 8, e71751. doi: 10.1371/journal.pone.0071751.

24. Fiester SE, Arivett BA, Schmidt RE, Beckett AC, Ticak T, Carrier MV, Ghosh R, Ohneck EJ, Metz ML, Jeffries MKS, Actis LA. Iron-regulated phospholipase C activity contributes to the cytolytic activity and virulence of *Acinetobacter baumannii*. PLoS ONE. 2016; 11:e0167068. doi: 10.1371/journal.pone.0167068.

25. Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS ONE. 2010; e10034.doi: 10.1371/journal.pone.0010034.

26. Fernando DM, Khan IUH, Patidar R, Lapen DR,Talbo G, Topp E, Kumar A. Isolation and Characterization of *Acinetobacter baumannii* recovered from Campylobacter Selective Medium. Frontiers in Microbiology; 2016; 7:1871.

27. Maravic A, Skocibusic, M, Fredotovic Z, Samanic I, Cvjetan S, Knezovic M, Puizina J. Urban riverine environment is a source of multidrug-resistant and ESBL-producing clinically important *Acinetobacter* Environ Sci Pollut Res. 2016; 23, 3525–3535.

28. Krizova L, Maixnerovaa M, Sedob O, Nemec A. *Acinetobacter bohemicus* nov.wide spread in natural soil and water ecosystems in the Czech Republic.*Systematic and Applied Microbiology*. 2014; 37, 467–473.

29. Goswami R, Mukherjee S, Rana VS, Saha DR, Raman R, Padhy PK, Mazumder S. Isolation and Characterization of Arsenic-Resistant Bacteria from Contaminated Water-Bodies in West Bengal, India. *Geo microbiology Journal*. 2015; 32, 17–26.

30. Xiong W, Sun Y, Zhang T, Ding X, Li Y, Wang M, Zeng Z. Antibiotics, Antibiotic Resistance Genes, and Bacterial Community Composition in Fresh Water Aquaculture Environment in China. Microb Ecol.

2015; 70:425–432.

31. Zhao J, Zhao X, Chao L, Zhang W, You T, Zhang J. Diversity change of microbial communities responding to zinc and arsenic pollution in a river of northeastern China. J Zhejiang Univ-Sci B (Biomed and Biotechnol). 2014; 15(7):670–680.

32. Chen TL, Lee YT, Kuo SC, Yang SP, Fung CP, Lee SD. Rapid identification of *Acinetobacter baumannii, Acinetobacter nosocomialis* and *Acinetobacter pittii* with a multiplex PCR assay. Journal of Medical Microbiology, 2014; 63, 1154–1159.

33. Chiang MC, Kuo SC, Chen YC, Lee YT, Chen TL, Fung CP. Polymerase chain reaction assay for the detection of *Acinetobacter baumannii* in endotracheal aspirates from patients in the intensive care unit. Journal of Microbiology, Immunology and Infection. 2011; 44, 106–110.

34. Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii.* Nat Rev Microbiol. 2007; 5, 939–951.

35. Park YK, Jung SI, Park KH, Kim DH, Choi JY, Kim SH, Ko KS. Changes in antimicrobial susceptibility and major clones of *Acinetobacter calcoaceticus– baumannii* complex isolates from a single hospital in Korea over 7 years. *Journal of Medical Microbiology*. 2012; 61(1) 71–79.

36. Anh NT, Thieu Nga TV, Tuan HM, Tuan NS, Dao Y, Chau NVV, Baker S, Duong HHT. Molecular epidemiology and antimicrobial resistance phenotypes of *Acinetobacter baumannii* isolated from patients in three hospitals in southern Vietnam. *Journal of Medical Microbiology*. 2017; 66:46–53.

37. Sileem AE, Said AM, Meleha MS. *Acinetobacter baumannii* in ICU patients: A prospective study highlighting their incidence, antibiotic sensitivity pattern and impact on ICU stay and mortality. Egyptian Journal of Chest Diseases and Tuberculosis. 2017; 66 693–698.

38. Berlau J, Aucken HM, Houang E, Pitt TL. Isolation of Acinetobacter spp including *baumannii* from vegetables: implications for hospital-acquired infections. Journal of Hospital Infection. 1999; 42: 201–204.

39. Ambrosi C, Scribano D, Aleandri M, Zagaglia C, Di Francesco L, Putignani L, Palamara AT. *Acinetobacter baumannii* Virulence Traits: A Comparative Study of a Novel Sequence Type with Other Italian Endemic International Clones. Front. Microbiol. 2017; 8:1977. doi: 10.3389/fmicb.2017.01977.

40. Eijkelkamp BA, Stroeher UH, Hassan KA, Paulsen IT, Brown MH. Comparative analysis of surface-exposed virulence factors of *Acinetobacter baumannii.* BMC Genomics. 2014; 15 (1020): 1-12.

41. Sato Y, Unno Y, Kawakami S, Ubagai T, Ono Y. Virulence characteristics of *Acinetobacter baumannii* clinical isolates vary with the expression levels of omps. Journal of Medical Microbiology. 2017; 66:203–212.

42. Antunes LCS, Imperi F, Carattoli A, Visca P. Deciphering the Multifactorial Nature of *Acinetobacter baumannii* PLoS ONE. 2011; 6(8): e22674. doi:10.1371/journal.pone.0022674.

43. Smith MG, Gianoulis TA, Pukatzki S, Mekalanos JJ, Ornston LN, Gerstein M, Snyder M. New insights into *Acinetobacter baumannii* pathogenesis revealed by high-density pyrosequencing and transposon mutagenesis. Genes and Development. 2007; 21:601–614.

44. McConnell MJ, Actis L, Pachon J. *Acinetobacter baumannii*: human infections, factors contributing to pathogenesis and animal models. FEMS Microbiol Rev. 2013; 37: 130–155.

45. Gaddy JA, Tomaras AP, Actis LA. The *Acinetobacter baumannii* 19606 *OmpA* protein plays a role in biofilm formation on abiotic surfaces and in the interaction of this pathogen with eukaryotic cells. Infection and Immunity. 2009; 77(8), 3150–3160.

46. Smani Y, Dominguez-Herrera J, Pachon J. Association of the outer membrane protein Omp33 with fitness and virulence of *Acinetobacter baumannii*. Journal of Infectious Disease. 2013; 208, 1561–1570. doi: 10.1093/infdis/jit386.

47. Choi CH, Hyun SH, Lee JY, Lee JS, Lee YS, Kim SA, Chae JP, Yoo SM, Lee JC. *Acinetobacter baumannii* outer membrane protein A targets the nucleus and induces cytotoxicity. Cell. Microbiol. 2008; 10: 309–319.

48. Lee JC, Koerten H, van den Broek P, Beekhuizen H, Wolterbeek R, van den Barselaar M, van der Reijden T, van der Meer J, van de Gevel J, Dijkshoorn L. Adherence of *Acinetobacter baumannii* strains to human bronchial epithelial cells. Res. Microbiol. 2006; 157:360–366.

49. Lee HW, Koh, YM, Kim J, Lee JC, Lee YC, Seol SY, Cho DT. Capacity of multidrug-resistant clinical isolates of *Acinetobacter baumannii* to form biofilm and adhere to epithelial cell surfaces. Clin. Microbiol. Infect. 2008; 14:49–54.

50. Lee JC, Oh JY, Kim KS, Jeong YW, Park JC, Cho JW. Apoptotic cell death induced by Acinetobacter baumannii in epithelial cells through caspase-3 activation. APMIS. 2001; 109:679–684.

51. Smani Y, Domnguez-Herrera J, Pachon J. Rifampin protects human lung epithelial cells against cytotoxicity induced by clinical multi and pandrug-resistant *Acinetobacter baumannii*. European Congress of Clinical Microbiology and Infectious Diseases. 2011; Vienna, Austria, April 2010.

52. Choi CH, Lee EY, Lee YC, Park TI, Kim HJ, Hyun SH, Kim SA, Lee SK, Lee JC. Outer membrane protein 38 of *Acinetobacter baumannii* localizes to the mitochondria and induces apoptosis of epithelial cells. Cellular Microbiology. 2005; 7, 1127–1138. doi: 10.1111/j.1462-5822.2005.00538.

53. Lee JS, Choi CH, Kim JW, Lee JC. *Acinetobacter baumannii* outer membrane protein A induces dendritic cell death through mitochondrial targeting. Journal of Microbiology, 2010; 48, 387–392. doi: 10.1007/s12275-010-0155-1.

54. Sugawara E, Nikaido H. *OmpA* is the principal nonspecific slow porin of *Acinetobacter baumannii.* Bacteriol. 2012; 194, 4089–4096. doi: 10.1128/JB.00435-12.

55. Smani Y, Fabrega A, Roca I, Sanchez-Encinales V, Vila J, Pachon J. Role of *OmpA* in the multidrug resistance phenotype of *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 2014; 58, 1806–1808. doi: 10.1128/AAC.02101-13.

56. Sibanda T, Chigor VN, Koba S, Obi CL, Okoh AI. Characterisation of the physicochemical qualities of a typical rural-based river: ecological and public health implications. Int. J. Environ. Sci. Technol. 2014; 11:1771–1780.

57. American Public Health Association, APHA. Standard Methods for the Examination of Water and Wastewater, 20th Ed.; (APHA): Washington, DC, USA. 2005.

58. Maugeri TL, Carbone M, Fera MT, Gugliandolo C. Detection and differentiation of *Vibrio vulnificus* in seawater and plankton f a coastal zone of the Mediterranean Sea. Research in Microbiology. 2006; 157(2), 194–200.

59. Chen TL, Siu LK, Wu RCC, Shaio MF, Huang LY, Fung CP, Lee CM, Cho WL. Comparison of one-tube multiplex PCR, automated ribotyping and intergenic spacer (ITS) sequencing for rapid identification of *Acinetobacter baumannii*. Clin Microbiol Infect; 2007; 13: 801–806.

60. Higgins PG, Wisplinghoff H, Krut O, Seifert H. A PCR-based method to differentiate between *Acinetobacter baumannii* and *Acinetobacter* genomic species 13TU. Clin Microbiol Infect. 2007; 13:1199–1201.

61. Thummeepak R, Kongthai P, Leungtongkam U, Sitthisak S. Distribution of virulence genes involved in biofilm formation in multi-drug resistant *Acinetobacter baumannii* clinical isolates. International Microbiology. 2016; 19:121-129 doi:10.2436/20.1501.01.270.

62. Momtaz H, Seifati SM, Tavakol M. Determining the prevalence and detection of the most prevalent virulence genes in *Acinetobacter baumannii* isolated from hospital infections. Int J Med Lab. 2015; 2(2):87–97.

63. Braun G, Vidotto MC. Evaluation of Adherence, Hemagglutination, and Presence of Genes Codifying for Virulence Factors of *Acinetobacter baumannii* Causing Urinary Tract Infection. Mem Inst Oswaldo Cruz, Rio de Janeiro, 2004; *99*(8): 839-844.

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	*Acinetobacter*genus	Delineated *Acinetobacter*species		Other species (ND)
			A. baumannii	***A. nosocomialis***	
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.

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

Table 3: The list of primers used for delineation of the presumptive *Acinetobacter* isolates into species.

Target gene	Primer name	Primer sequence(5′-3′)	Amplicon size (bp)	Reference
gyrB	P-Ab-ITSF	CATTATCACGGTAATTAGTG	208	[59]
	P-Ab-ITSR	AGAGCACTGTGCACTTAAG		
gyrB	sp4F	CACGCCGTAAGAGTGCATTA	294	[60]
	sp4R	AACGGAGCTTGTCAGGGTTA		

Table 4: PCR primers for determination of virulence genes in *Acinetobacter* isolates

e	Virulence factor	Primer sequences	Amplicon size	Tm (°C)	Reference
draBC	Dr fimbriae	GCTGGGCAGCAAACTGATAACTCTC CATCAAGCTGTTTGTTCGTCCGCCG	750	63	[63]
4	Exo-polysaccharide	AGCAAGTGGTTATCCAATCG ACCAGACTCACCCATTACAT	451	50	[61]
H	Type 1 fimbriae	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	508	63	[63]
pA	Outer membrane protein	CGCTTCTGCTGGTGCTGAAT CGTGCAGTAGCGTTAGGGTA	531	50	[61]
	Pathogenicity-associated island	GGACATCCTGTTACAGCGCGCA TCGCCACCAATCACAGCCGAAC	930	50	[63]
focDE	S fimbriae	CTCCGGAGAACTGGGTGCATCTTAC CGGAGGAGTAATTACAAACCTGGCA	410	63	[63]
	Serum resistance	GGTGTGGTGCGATGAGCACAG CACGGTTCAGCCATCCCTGAG	290	63	[63]

Figures

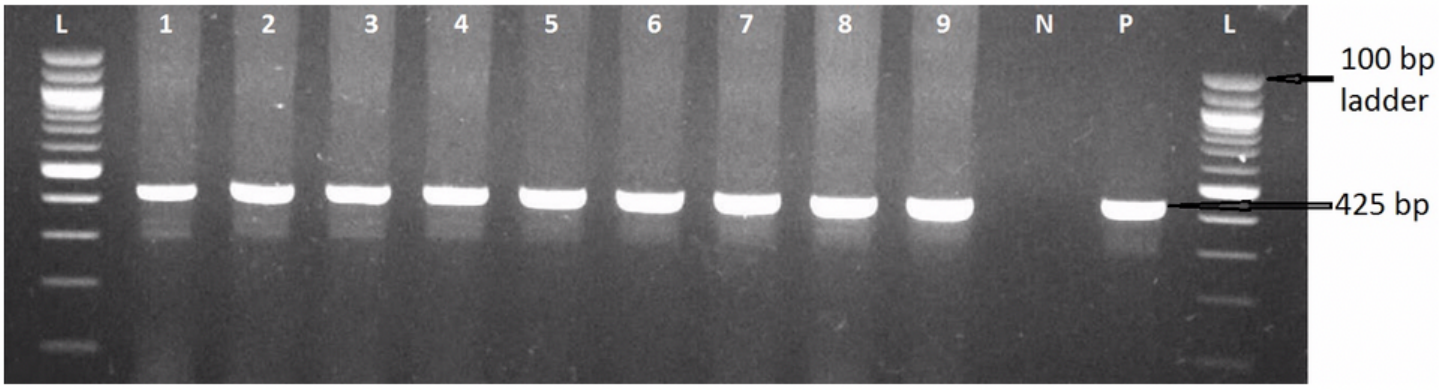

Figure 1

PCR analyses resolved by gel electrophoresis showing confirmed Acinetobacter spp. targeting the recA gene at 425 bp. L= DNA Ladder (100bp); Lane 1 to 9 = Selected Acinetobacter isolates; N= Negative control; P=Positive control (A. baumannii, DSM Number: 102929).

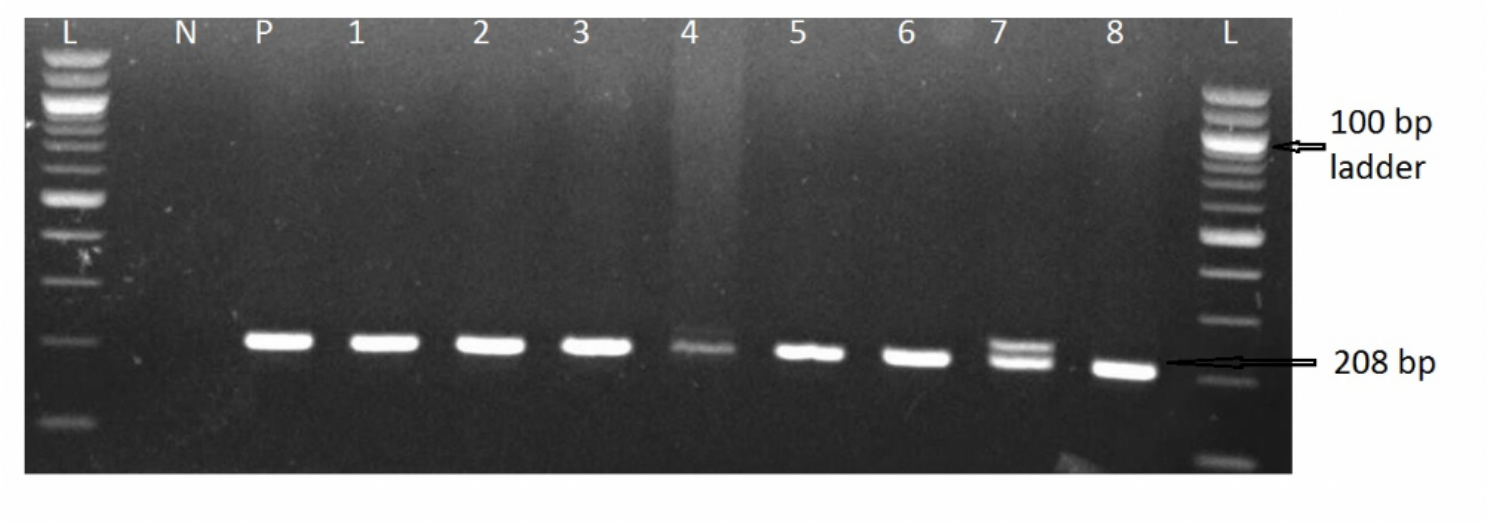

Figure 2

PCR analyses resolved by gel electrophoresis showing confirmed A. baumannii targeting the specific primer Ab-ITS gene at 208 bp. L= DNA Ladder (100bp); N= negative control, P= positive control (A. baumannii, DSM 102929); Lanes 1 to 8= selected A. baumannii samples.

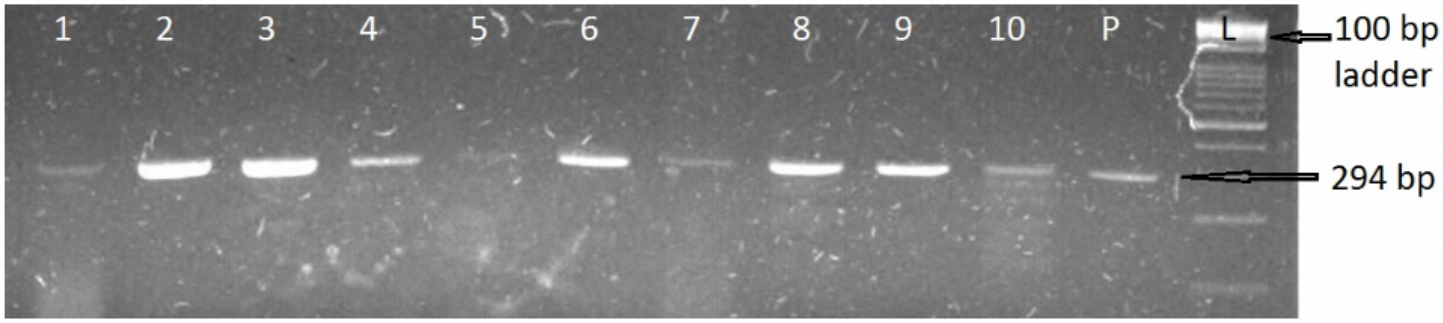

Figure 3

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

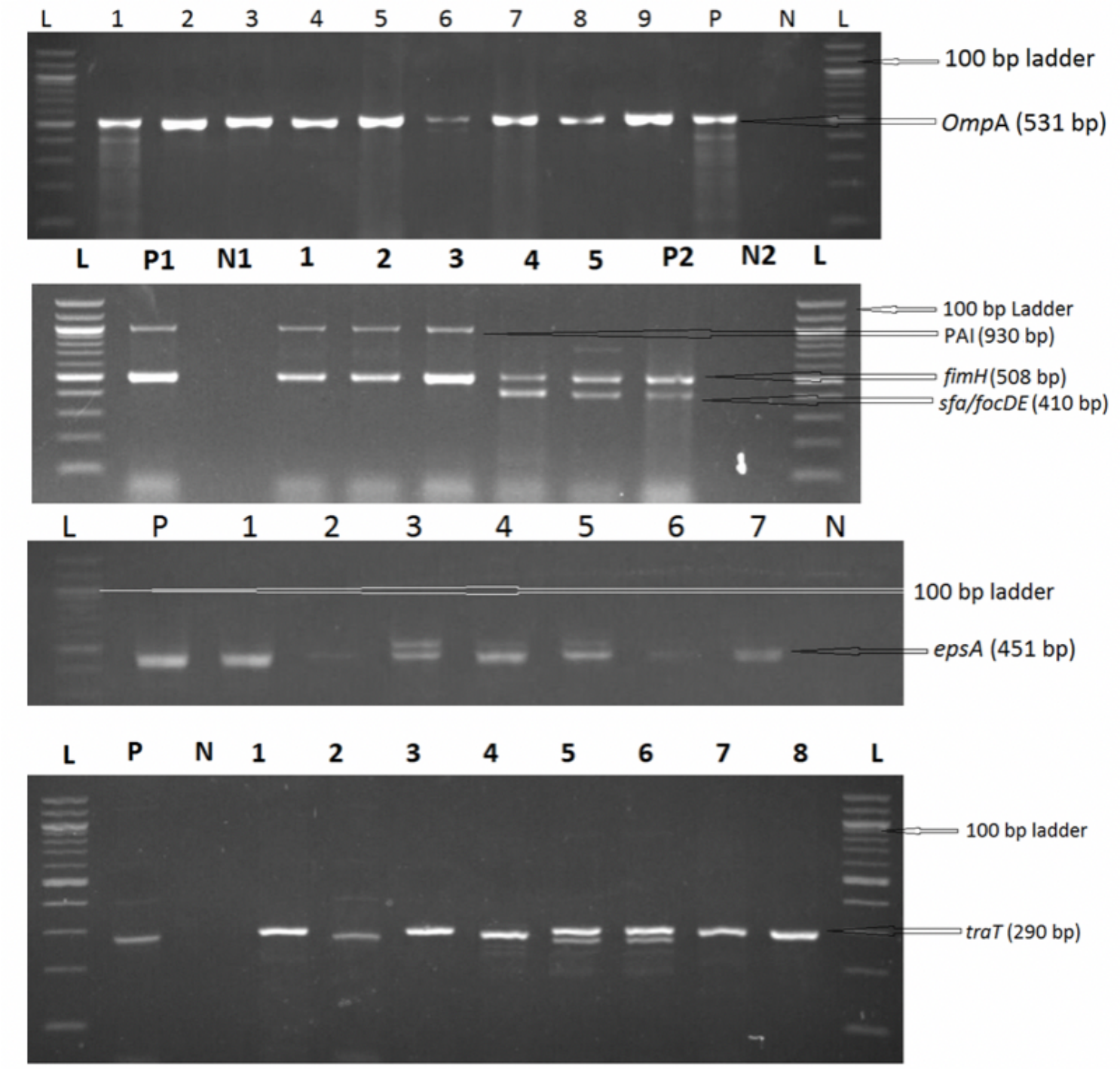

Figure 4

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).

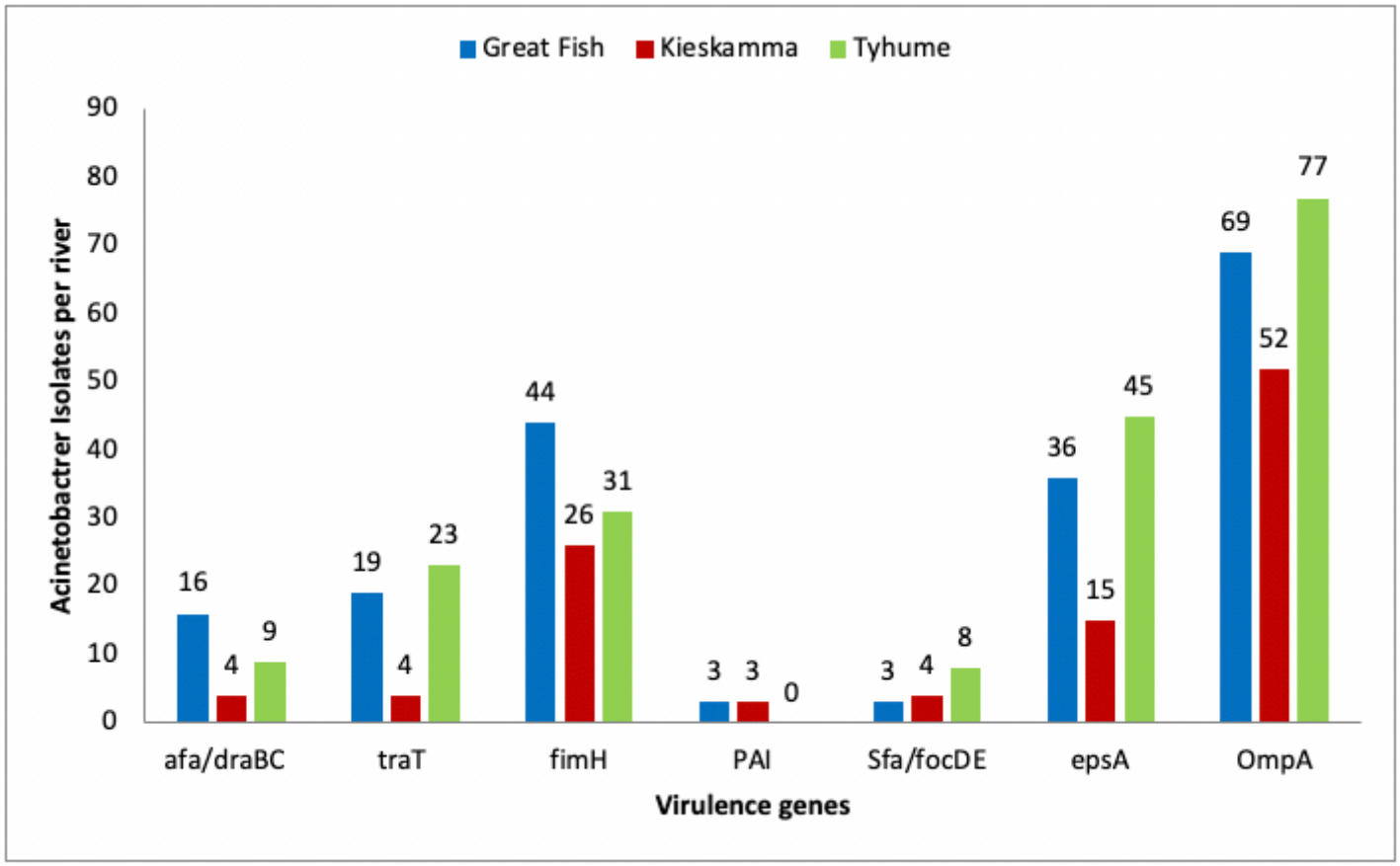

Figure 5

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