Original article

Prevalence and molecular analysis of multidrug-resistant *Acinetobacter baumannii* in the extra-hospital environment in Mthatha, South Africa

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ABSTRACT

Introduction: The presence of *Acinetobacter baumannii* outside hospitals remains unclear. This study aimed to determine the prevalence of multidrug-resistance (MDR) *A. baumannii* in the extra-hospital environment in Mthatha, South Africa and to investigate the frequency of carbapenemase-encoding genes.

Material and Methods: From August 2016 to July 2017 a total of 598 abattoir samples and 689 aquatic samples were collected and analyzed presumptively by cultural methods for the presence of *A. baumannii* using CHROMagar™ *Acinetobacter* medium. Species identification was performed by autoSCAN-4 (Dade Behring Inc., IL) and confirmed by the detection of their intrinsic bla_{OXA-51} gene. Confirmed MDR *A. baumannii* isolates were screened for the presence of carbapenemase-encoding genes, ISAba1 insertion sequence and integrase *intI1*.

Results: In total, 248 (19.3%) *Acinetobacter* species were isolated. *Acinetobacter. baumannii* was detected in 183 (73.8%) of which 85 (46.4%) and 98 (53.6%) were recovered from abattoir and aquatic respectively. MDR *A. baumannii* was detected in 56.5% (48/85) abattoir isolates and 53.1% (52/98) aquatic isolates. Isolates showed high resistance to antimicrobials most frequently used to treat *Acinetobacter* infections such as piperacillin/tazobactam; abattoir (98% of isolates resistant), aquatic (94% of isolates resistant), ceftazidime (84%, 83%), ciprofloxacin (71%, 70%), amikacin (41%, 42%), imipenem (75%, 73%), and meropenem (74%, 71%). All the isolates were susceptible to tigecycline and colistin. All the isolates carried $bla_{OXA-51-like}$. The bla_{OXA-23} was detected in 32 (66.7%) abattoir isolates and 11 (21.2%) aquatic isolates. The

$bla_{OXA-58-like}$ was positive in 7 (14.6%) and 4 (7.7%) abattoir and aquatic isolates, respectively. Both groups of isolates lacked $bla_{OXA-24-like}$, $bla_{IMP-type}$, $bla_{VIM-type}$, bla_{NDM-1}, bla_{SIM}, bla_{AmpC}, IS$Aba1$ and $inI1$. Isolates showed high level of Multiple Antibiotic Resistance Index (MARI) ranging from 0.20-0.52.

Conclusion: Extra-hospital sources such as abattoir and aquatic environments may be a vehicle of spread of MDR *A. baumannii* strains in the community and hospital settings.

Introduction

Acinetobacter baumannii has emerged over the few last decades as a cause of healthcare-associated infections with this organism associated with increased patient morbidity, mortality, and treatment costs.[1–3] Its clinical significance has been propelled by its remarkable ability to upregulate or acquire resistance determinant making it one of the organisms threatening the current antibiotic era.[4,5] The possession of this array of resistance mechanisms have made this organism to be able to resist almost all available antibiotics in hospital environment and has position the organism as important candidate for the evaluation of reservoirs of antibiotic resistance in the environment or even in human subjects.[6] *Acinetobacter. baumannii* have the ability to survive in both moist and dry conditions for many weeks or even months, hence this organism is widely distributed in natural and nosocomial environments, human skin, as well as mucosal surfaces facilitating its spread.[7–9] The cross-transmission of this organism from patient to patient and the possibility of outbreak extension by patient transfer have been demonstrated.[10] Despite hospital ecology being intensively studied, its ecology outside the hospital remains unclear or poorly defined impeding effective prevention of transmission.[2,11] Several investigators have suspected that the survival of *A. baumannii* in the environment (in particular in water) could contribute to the transmission of the organism during outbreaks.[12] It is well established that members of the genus *Acinetobacter* is ubiquitous but difficulty in unequivocally identify this organism has made the ubiquity in nature of *A. baumannii* a widespread misconception.[1,9] Therefore the existence of an extra-hospital reservoir of *A. baumannii* and the implication of this potential reservoir in the occurrence of certain infections due to this organism are still controversial.[1,2] Additionally, the prevalence and antimicrobial resistance of *A. baumannii* outside the hospital setting has been poorly investigated.[13] There seems to be a continuous influx of novel strains into the clinical setting with the potential of new infectious features.[11] Studies have shown that infections caused by extra-hospital strains of *A. baumannii* are responsible for community-acquired infections mainly in tropical and subtropical areas.[5,14] This suggests a source of this pathogen outside of the hospital and therefore the significance of environmental isolates in the epidemiology of *A. baumannii* is of a great concern worldwide. There is no clear evidence about the way of introduction of *A. baumannii* into hospital environment, its propagation from hospital settings to the natural environment and its natural habitat outside hospitals.[15] Also unlike clinical strains, there are limited reports using molecular analysis to explore the extra-hospital epidemiology of *A. baumannii*.[11,16] The presence of *A. baumannii* in the aquatic environment raises the issue of public health risk since people are in constant contact with the aquatic environment.[17] Hospital waste water is discharged into municipal sewage system without prior treatment, contributing to widespread contamination of natural waters with emerging pathogenic bacteria that can carry MDR genes.[18] In the aquatic environment, bacteria interact with various organisms, some of which are fish used for consumption as well as commercial or recreational purposes.[19] Most of the antibiotics used in the hospitals end up contaminating our local dams. The indiscriminate use of antibiotics in livestock production as growth promoters or to control infectious diseases and the treatments have been linked with the dissemination of resistant bacteria which can be transferred to people via food, direct contact with infected animal or through the abattoir. In a study conducted in Scotland, pigs and cattle slaughtered for human consumption and coming from different farms were sampled (faeces, skin, nostril and ear) for *A. baumannii* isolation. The prevalence of *A. baumannii* carriage was 1.2%. The 16 *A. baumannii* isolated were grouped into three different clusters, but had different pulsed-field gel electrophoresis (PFGE) patterns compared with human isolates of the three major European clones Eci, ECII, and ECIII.[20] Recent reports have also described the presence of carbapenem-resistant *A. baumannii* in animals. The OXA-23 carbapenemase has been found in *Acinetobacter* from cattle, horses, and cats.[21–23] The NDM-1 has also been reported in *Acinetobacter* spp. from food animals (chicken and pig farms) in China.[24,25] However, knowledge about carbapenemase-producing *A. baumannii* of environmental origin (aquatic and abattoir in case of this study) remains very limited, making it difficult to assess its impact on public health. In South Africa, *A. baumannii* has been isolated from hospital environments; nevertheless only few studies have reported the presence of *A. baumannii* in the aquatic environment, meat of food animals and the environment where these meats come from. These areas are a source of water and food and therefore can be of transmission of this organism into hospital settings or may horizontally transfer resistance genes to other organisms.

Study objectives

Based on these premises, the present study was carried out to determine the prevalence of MDR *A. baumannii* in aquatic and

abattoir environments, their susceptibility to antimicrobials and to determine the frequency of carbapenemase-encoding genes.

Material and methods

Study design and settings

This was a Cross-sectional prospective descriptive study conducted between the period of August 2016 and July 2017 at:

1 The Umzikantu red meat abattoir, a private abattoir located in South Ridge Park, O.R. Tambo district municipality in Mthatha, Eastern Cape. This red meat abattoir is a type E5 abattoir. It operates as an abattoir and meat wholesalers and it is the only operational abattoir in Mthatha. Abattoirs differ greatly in the scale of their operations. Using the EU definition of livestock units, the scale is graded according to the number of beast they are designed to handle (without some health risk) per week as follows: E: - to slaughter five units, D: - to slaughter 15 units, C: - to slaughter 50 units, B: - to slaughter 200 units, and A: - to slaughter 1000 units (exporting type). In its basic form one EU Livestock Unit (ELU) equals: one cow beast, two calves, five pigs or 10 sheep.[26]

2 The Mthatha dam (coordinates: 31°33′2″S 28°44′24″E), an earth-fill type dam which impounds the Mthatha river at a location about eight kilometers upstream of the city of Mthatha in the OR Tambo District Municipality of the Eastern Cape Province. The dam was established in 1977 and serves mainly for municipal and industrial purposes. Its hazard potential has been ranked high. The storage capacity of the Dam is 253,674,000 cubic meters. It serves as the main source of raw water supply (65.9 mL/day) for the Thomhil WTW which treats and supplies water to the city and its environs with a total population of about 750,000.

Sample collection from abattoir environment

Five hundred and ninety-eight samples were recovered from the hides, from structural and work surfaces (equipment, floors, doors, knives, saws, handling panels from the slaughter area) within the abattoir and from carcasses and meat at all stages of processing (from flaying to evisceration, splitting and cooling). At each site, an area of 10 cm^2 was sampled using a sterile swab moistened with physiological saline. Animal samples were also collected from recently slaughtered animals destined for human consumption. Carcasses were sampled by systematic random sampling technique. Swabs were taken according to the method described by the international organization for standardization.[27] The abdomen (flank), thorax (lateral), crutch, breast (lateral), were the sampling sites. Swabbing was performed approximately six hours after cleaning and disinfection and prior to slaughter activities in the abattoir. The samples were cooled and transported to the laboratory for plating within 10 h after sampling. For meat samples, 25 g of meat were cut aseptically into very small pieces and homogenized by using a stomacher bag

(Interscience, Saint Nom, France) and then suspended in sterile water.

Sample collection from aquatic environment

Overall, 689 samples were collected from the Mthatha dam. The dam was sampled five times within the study period and samples were collected using aseptic techniques in the mornings (between 08:00 and 10:00 h local time). Duplicate water samples were collected at margins and in the middle of the dam, about 15 cm down the water surface in 100 ml sterilized bottles. The collected water samples were placed in a cooler box with temperature maintained between 4 and 10°C using ice packs. These samples were immediately transported to the Walter Sisulu University laboratory and subjected to serial ten-fold dilutions in sterile distilled water. Forty-nine fishes belonging to different species were collected aseptically and immediately transported in a thermal bag to the laboratory where they were sacrificed. Samples from skin, gills, intestines, and eyes were collected. The body surface and gills were carefully washed, at first under the stream of tap water and then using 70% ethyl alcohol to remove normal external bacterial flora. Then, the fish were disinfected with 70% ethanol and after opening the body cavity, samples from the kidneys and intestine were taken. The samples were diluted 1:1 in PBS and homogenized.

Bacterial cultivation and identification

Samples were presumptively identified by direct plating on Chromogenic CHROMagar™ *Acinetobacter* (CHROMagar™; Paris, France) with supplement Ref. CR102 which allows the growth of carbapenem-resistant isolates of *Acinetobacter* species, incubated at 37 °C in aerobic conditions and examined after 24 h for the growth of typical red colonies of *Acinetobacter* species. CHROMagar™ *Acinetobacter* was prepared according to the manufacturer's description. Cefsulodin sodium salt hydrate (Sigma-Aldrich) was added at 15 mg/L to suppress the growth of *Pseudomonas* and *Aeromonas* species. Gram-positive bacteria and yeasts are inhibited. Confirmation of presumptive colonies was performed by inspection of colony morphology on the separate plates. The plates were examined for the growth of typical red colonies of *Acinetobacter* species. One typical colony representative of each type of morphology and shape was further sub-cultured on tripticase soy agar (TSA) and characterized based on phenotypic tests: Gram-stain, catalase and oxidase tests. Gram-negative coccobacilli, oxidase-negative, and catalase-positive isolates were presumptively identified to the genus *Acinetobacter*. Species identification were carried out using Gram-negative ID type 2 panel (REF. B1017-27) of the MicroScan autoSCAN-4 automated System (Dade Behring Inc., Deerfield, IL). A high percentage (≥95%) was utilized as the acceptance criterion for identification by MicroScan. Suspected colonies were also further verified using the *Acinetobacter* specific primer set Ac436 F and Ac676 r to amplify the 16S rRNA gene. Confirmation of *A. baumannii* isolates was carried out by polymerase chain reaction analysis of the presence of inherent *Bla*$_{OXA-51-like}$ genes. Isolates lacking the *Bla*$_{OXA-51-like}$ gene were investigated further by sequence analysis of the 16S rRNA gene. Primers were ordered from TIB Molbiol, Germany. All the strains were freshly suspended in skim

milk (Merck, Germany) containing 15% glycerol in sterile glass, screw-capped vials and stored at -80 °C until further use.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by MicroScan autoSCAN-4 System. The antibiotics tested were amikacin (AMK), amoxicillin/clavulanic acid (AMC), ampicillin/sulbactam (AMS), ampicillin (AMP), cefazolin (CEP), cefepime (FEP), cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO), cefuroxime (CXM), cefoxitin (FOX), cephalothin (CEPH), ciprofloxacin (CIP), gentamicin (GEN), imipenem (IMP), meropenem (MEM), levofloxacin (LVX), nitrofurantoin (NIT), tetracycline (TEC), tobramycin (TOB), trimethoprim/sulfamethoxazole (SXT), piperacillin/tazobactam (TZP), piperacillin (PRL), colistin (CST), and tigecycline (TGC) according to the CLSI guidelines v27.[28] Non-susceptibility was defined as the combination of resistance and intermediate resistance. MDR *A. baumannii* isolates were defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.[29] *Escherichia coli* (ATCC #25922) and *Pseudomonas aeruginosa* (ATCC #27853) were included as quality control strains.

Multiple antibiotic resistance index (MARI)

The MARI was calculated and interpreted according to Krumperman (1983) as the ratio of the number of antibiotics to which the isolates were resistant (a) to the total number of antibiotics to which the isolates were exposed (b), i.e: MARI = a/b.

The MARI of individual *A. baumannii* from the two different sources was calculated. Bacteria having MARI (>0.2) originate from a high risk source of contamination where several antibiotics are used. MARI value of ≤0.2 indicates strain originated from sources where antibiotics are seldom or never used.[30]

Investigation of carbapenemase-encoding genes, ISAba1 and intI1 by PCR

Genomic DNA from an overnight culture on tryptic soy broth of *A. baumannii* was extracted using the MagNaPure Compact® nucleic acid isolation kit I (REF. 03730964001, Roche diagnostics, Mannheim, Germany) according to the manufacturer's protocol. The presence of the genes encoding Ambler class D Serine-Carbapenemase ($bla_{OXA-51-like}$, $bla_{OXA-23-like}$, $bla_{OXA-24-like}$, and $bla_{OXA-58-like}$), Ambler class B Metallo-β-lactamases (bla_{IMP-1}, bla_{VIM}, bla_{SIM} and bla_{NDM-1}) and Ambler class C bla_{AmpC} were analyzed by PCR. IS*Aba1* and *intI1* were also determined. The presence of the IS*Aba1* upstream $bla_{OXA-23-like}$ and $bla_{OXA-51-like}$ genes to promote gene expression were performed using IS*Aba1* forward/$bla_{OXA-23-like}$ reverse or $bla_{OXA-51-like}$ reverse primers.[31] The details of primer sequences used for PCR amplification of the resistant genes are listed in Table 1.

Ethical considerations

Ethical approval for this project was obtained from the Walter Sisulu University Research Ethics and Biosafety Committee [Reference number: 019/2016]. Also written permission was sought from the authorities of the two study sites. For the protection of animals used for scientific purposes the experiments described complied with the guidelines of the European Union Council http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm (Directive 2010/63/EU).

Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS, IBM, version 23.0 Armonk, NY). The frequency of some parameters including *A. baumannii* susceptibility and also resistant genes were compared between abattoir and aquatic *A. baumannii* by Chi-square or Fisher's exact test. P-values <0.05 were considered as significant.

Results

Antimicrobial susceptibility testing and MARI

A total of 1287 samples were collected from the abattoir and aquatic sources from which 248 (19.3%) *Acinetobacter* species were isolated. *A. baumannii* was the predominant species (n = 183, 73.8%) of which 85 (46.4%) and 98 (53.6%) were recovered from abattoir and aquatic, respectively. Of these a total of 100 MDR *A. baumannii* isolates, including 56.5% (48/85) abattoir and 53.1% (52/98) aquatic, were selected for analysis. Table 2 shows the distribution of the identified *Acinetobacter* species according to the sources of samples. All MDR isolates showed a susceptible phenotype in response to colistin and tigecycline. Among abattoir isolates, all (100%) were resistant to trimethoprim-sulfamethoxazole, and >70% were resistant to cephalosporins (ceftazidime, cefazolin, cephalothin, cefotaxime, ceftriaxone, cefepime, cefuroxime, and cefoxitin), gentamycin and ciprofloxacin. Carbapenem resistant was 75% against imipenem and 74% against meropenem. However, there were considerable differences in resistance rates and patterns between the isolates recovered from the two study areas. Chi-square test revealed that there were no significant differences in resistance rates among different sources of isolates (p < 0.253). The resistance rates against imipenem, meropenem, ciprofloxacin, and gentamycin were 73%, 71%, 70%, and 75%, respectively, in isolates recovered from aquatic source, whereas resistance rates were 57–94% against other antimicrobial agents except for amikacin (42%). Fig. 1 shows the resistance characteristics of these isolates. The *A. baumannii* exhibited 25 antibiotic resistant patterns with high level of MARI (>0.2) ranging from 0.20–0.52.

Mean MARI of isolates from both sources are shown in Fig. 2. The majority of the *A. baumannii* from the abattoir source (31 isolates, 64.6%) were resistant to at least eight antibiotics

Table 1 – List of primers used for PCR amplification of resistant genes.				
Gene	Oligonucleotide sequence	Fragment size (bp)	Location	References
16SrRNA	F: 5'TTT AAG CGA GGA GGA GG3' R: 5'ATT CTA CCA TCC TCT CCC3'	240	16SrRNA	[32]
OXA-23-like	F: 5'GATCGGATTGGA-GAACCAGA3' R: 5'ATTTCTGACCGCATTTCCAT3'	501	bla_{OXA-23}	[33]
OXA-24-like	F: 5'GGTTAGTTG GCC CCC TTA AA3' R: 5'AGTTGAGC-GAAAAGGGGATT3'	246	bla_{OXA-24}	[33]
OXA-51-like	F: 5'TAATGCTTT GATCGG CCT TG3' R: 5'TGGATTGCACTT CAT CTT GG3'	353	bla_{OXA-51}	[33]
OXA-58-like	F: 5'AAGTATTGGGGCTTGTGCTG-3' R: 5'CCCCTCTGCGCTCTACATAC3'	599	bla_{OXA-58}	[33]
IMP-1	F: 5'GATGGTATGGTGGCTCTTGT3' R: 5'TTAATTTGCCGGACTTAGGC3'	448	Bla_{IMP}	[34]
NDM-1	F: 5'ATTAGCCGCTGCATTGAT3' R: 5'CATGTCGAGATAGGAAGTG3'	154	bla_{NDM}	[35]
VIM-like	F: 5'ACTCACCCCCATGGAGTTTT3' R: 5'ACGACTGAGCGATTTGT-GTG3'	815	bla_{VIM}	[36]
SIM-1-like	F: 5'TAATGCTTT GATCGG CCT TG3' R: 5'TGGATTGCACTT CAT CTT GG3'	353	bla_{SIM}	[33]
AmpC	F: ACAGAGGAGCTAATCATGCG R: GTTCTTTTAAACCATATACC	1243	bla_{ampC}	[37]
ISAba1	F: 5-CACGAATGCAGAAGTTG-3 R: 5-CGACGAATACTATGACAC-3	599	ISAba1	[38]
ISAba1/$bla_{OXA-23-like}$	F: AATGATTGGTGACAATGAAG R: ATTTCTGACCGCATTTCCAT	1433	ISAba1/bla_{OXA-23}	[38]
ISAba1/$bla_{OXA-51-like}$	F: AATGATTGGTGACAATGAAG R: TGGATTGCACTTCATCTTGG	1252	ISAba1/bla_{OXA-51}	[31]
intI1	F: 5'CAG TGG ACA TAA GCC TGT TC3' R: 5'CCC GAC GCA TAG ACT GTA3'	160	Integrase gene (intI1)	[39]

whilst the majority of *A. baumannii* from the aquatic source (21 isolates, 40.4%) were resistant to six antibiotics (Table 3).

Molecular detection of carbapenemase-encoding genes in *A. baumannii*

The two groups of MDR isolates were analyzed for carbapenemase-encoding resistance genes ($bla_{OXA-23-like}$, $bla_{OXA-24-like}$, $bla_{OXA-51-like}$, $bla_{OXA-58-like}$, bla_{IMP-1}, bla_{SIM}, bla_{VIM}, bla_{ampC} and bla_{NDM-1}). The bla_{OXA-51} is intrinsically found in *A. baumannii* and was detected in all abattoir and aquatic isolates which is consistent with the $bla_{OXA-51-like}$ gene as an intrinsic carbapenemase. A total of 32 (66.7%) of the 48 abattoir isolates carried bla_{OXA-23}, whereas 11 (21.2%) of the 52

aquatic isolates were bla_{OXA-23} positive. The $bla_{OXA-58-like}$ was detected in seven (14.6%) and four (7.7%) abattoir and aquatic isolates respectively. There were no $bla_{OXA-24-like}$, $bla_{IMP-type}$, $bla_{VIM-type}$, bla_{NDM-1}, bla_{SIM}, and bla_{ampC} genes among both groups of isolates. All isolates lacked ISAba1 and ntI1. According to Chi-square test, there were significant differences in the detection of resistant genes among different sources of isolates (p < 0.001).

Discussion

Over the past two decades, *Acinetobacter* species have become virulent pathogens, responsible for nosocomial infections

Table 2 – Distribution of the identified *Acinetobacter* species according to the sources of samples.

Acinetobacter species isolated	Total no. of isolates	Abattoir isolates		Aquatic isolates	
		Source	Number of isolates found	Source	Number of isolates found
MDR *A. baumannii*	100	Floors	5	Sediment	17
		Doors	1	Water Sample	15
		Knives	9	Gills	5
		Saws	7	Intestines	3
		Handling Panels	2	Eyes	5
		Slaughter Area	4	Skin	7
		Abdomen (Flank)	7		
		Thorax (Lateral)	3		
		Crutch	2		
		Breast (Lateral)	8		
		Total	**48**	**Total**	**52**
Non-MDR *A. baumannii*	83	Floors	8	Sediment	9
		Doors	4	Water Sample	15
		Knives	9	Gills	6
		Saws	6	Intestines	3
		Handling Panels	4	Eyes	5
		Slaughter Area	5	Skin	8
		Abdomen (Flank)	1		
		Total	**37**	**Total**	**46**
Total A. baumannii strains (MDR and Non-MDR)	183		85		98
Acinetobacter lwoffii	28	Floors	2	Sediment	3
		Doors	3	Water Sample	2
		Knives	3	Gills	1
		Saws	2	Intestines	3
		Handling Panels	2	Eyes	1
		Slaughter Area	4	Skin	1
		Abdomen (Flank)	1		
		Total	**11**	**Total**	**13**
Acinetobacter calcoaceticus	24	Floors	3	Sediment	4
		Doors	4	Water Sample	2
		Saws	2	Gills	1
		Handling Panels	1	Intestines	2
		Slaughter Area	1	Eyes	3
				Skin	3
		Total	**11**	**Total**	**13**
Acinetobacter pittii	13	Floors	2	Sediment	2
		Doors	1	Water Sample	1
		Handling Panels	1	Intestines	4
		Slaughter Area	1	Skin	1
		Total	**5**	**Total**	**8**
Total	**248**		**118**		**130**

Table 3 – Multiple antibiotic resistance among *A. baumannii* isolates from sampling sites (n = number of isolates).

Sampling sites	Five antibiotics (n) (%)	Six antibiotics (n) (%)	Seven antibiotics (n) (%)	>Seven antibiotics (n) (%)
Abattoir	2 (4.2%)	10 (20.8%)	5 (10.4%)	31 (64.6%)
Aquatic	7 (13.5%)	21 (40.4%)	6 (11.5%)	18 (34.6%)

and outbreaks, particularly in intensive care units and high dependency units (HDUs).[2] Majority of published studies have concentrated on the hospital epidemiology of these organisms[2] and animal health care settings making it difficult to demonstrate the extra-hospital origin of *A. baumannii*.

The intensive use of antibiotics over the last few decades in human and as growth promoting and as prophylactic agents in livestock have resulted in serious environmental and public health problems, since this enhances antimicrobial selective pressure. Also anthropological activities have
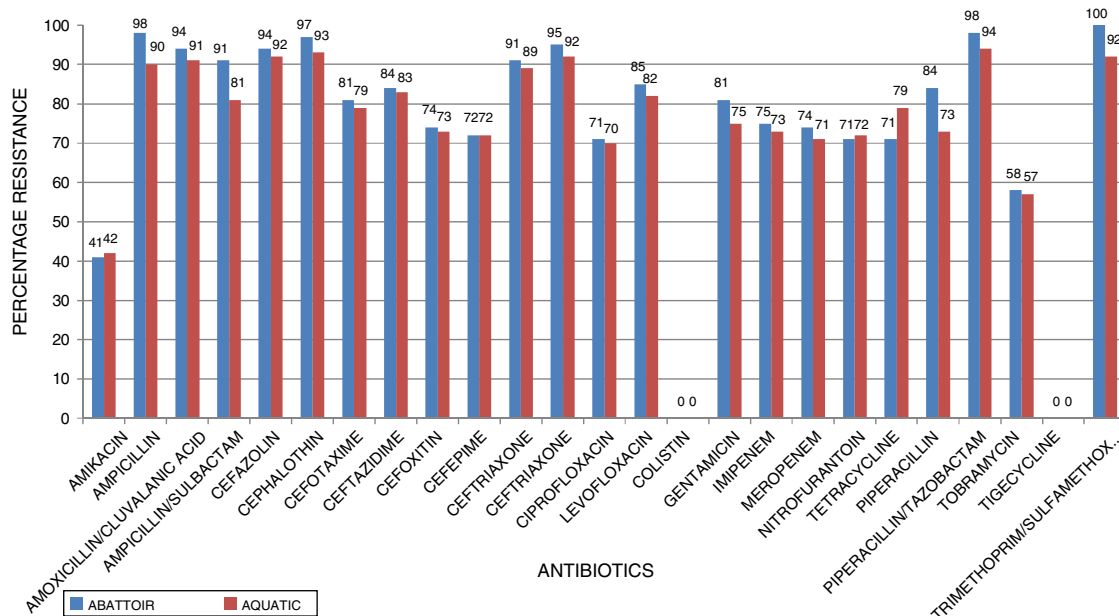
Fig. 1 – **Antimicrobial susceptibility profiles of *A. baumannii* isolates from the two groups of isolates (aquatic and abattoir).**
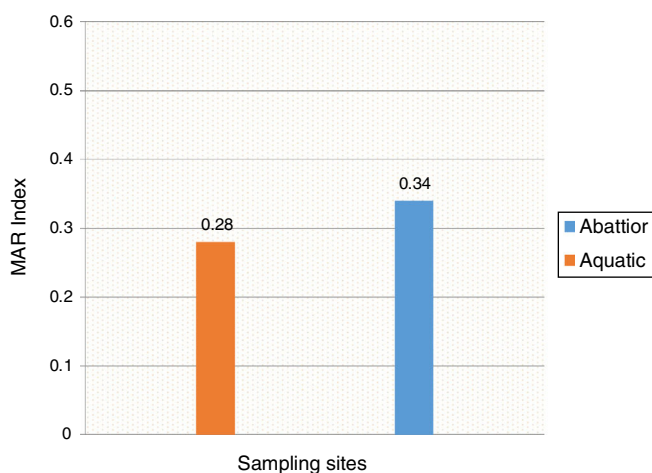

Fig. 2 – **Mean Multiple Antibiotic Resistance Index (MARI) values in Abattoir and Aquatic isolates.**

great impacts on our local dams. The antibiotic residues concentration increases in hospital and industrial effluents and these end up in our local dams leading to the development of antibiotic-resistant bacteria. Gros et al., studied the antibiotic concentrations in aquatic environments and the median concentrations in surface and ground water were reported as 0.030 and 0.071 μgL^{-1}, respectively.[40] These concentrations are above the MIC/MBC for all antibiotics, according to the British Society for Antimicrobial Chemotherapy.[41] *A. baumannii* isolated from various environmental locations has been linked with nosocomial spread.[42] The resistant bacteria from the extra-hospital environments may be transmitted to humans, in whom they cause disease that cannot be treated by conventional antibiotics. In aquatic environment two hypotheses

can arise from the detection of *A. baumannii*: (1) water is a normal habitat of *A. baumannii,* or (2) the presence of the bacterium results from human or animal contamination. To our knowledge, this is the first study investigating the molecular mechanisms accounting for the multidrug-resistance observed in *A. baumannii* recovered from abattoir and aquatic samples in the Eastern Cape Province of South Africa.

In the current study, the prevalence of *A. baumannii* was 14.2% in abattoir and 14.0% in aquatic samples collected. Of these, MDR *A. baumannii* was recovered from 56.5% (48/85) abattoir isolates and 53.1% (52/98) aquatic isolates and were analyzed. Therefore, our study evidenced the extra-hospital presence of *A. baumannii* in Eastern Cape Province of South Africa. This is in agreement with a previous study that found 51.2% (85/166) from meat samples to be MDR,[9] i.e. acquired resistance to at least one agent in three or more antimicrobial categories among the eight different classes of antibiotics used to define MDR.[29] In contrast, a study conducted by Stenstrom et al. in Nkonkobe Municipality of the Eastern Cape Province, South Africa, found that the prevalence of *Acinetobacter calcoaceticus* complex from soil and water samples in Alice town was 41%.[6] Also previous studies in Senegal, Scotland, and La Reunion Island isolated *A. baumannii* as animal colonizers in 5.1%, 1.2%, and 6.5%, respectively.[5,13,20] The study also revealed the quantitative changes in antibiotic resistance at two sampling sites. In general, susceptibility testing showed that 98% in abattoir isolates and 90% in aquatic isolates were resistant to ampicillin which is usually a non-effective antibiotic for the treatment of *Acinetobacter* infections. A high incidence of ampicillin resistance in aquatic environments was reported.[43] High resistance to trimethoprim/sulfamethoxazole in abattoir (100%) and aquatic isolates (92%) is a result of the inherent resistant of *A. baumannii* to this compound through the target protein, dihydrofolate reductase, which has a low affinity for the drug[44] and also

to selective pressure caused by the use of these antimicrobial agent for treatment of infections in cattle. The MARI is a good risk assessment tool and its value (nominally 0.20) has been applied to differentiate low- and high-risk regions where antibiotics are overused.[45] The present study showed MARI ranging from 0.20 to 0.52 (mean MARI; aquatic, 0.28 and abattoir, 0.34) which indicates that the isolates emerged from high-risk sources of contamination and also gives an idea of the number of *A. baumannii* isolates showing antibiotic resistance in the risk zone of the susceptibility study. This indicates that the study areas where these isolates came from were exposed to an environment of high-risk contamination from a region or area where there is high antibiotic use,[30] probably from hospital and industrial effluents which find their way into aquatic environment or the indiscriminate use of antibiotics in the livestock slaughtered at the abattoir. This can lead to high antibiotic resistance selective pressure.[46] The high resistance rate to ciprofloxacin, gentamycin, and tetracycline was not expected. These antimicrobials may have been used in these animals either as growth promoter, prophylactic or for treatment in case of abattoir isolates and also ending in aquatic environment. This result reflects the use of antimicrobial agents in livestock in South Africa, as tetracycline has long been the antimicrobial most commonly used in livestock, accounting for over 30% of the total amount of antimicrobial consumption.[47] Furthermore the high resistant rate to cephalosporins may be because this antibiotic is frequently used in hospitals and might have found their way into the aquatic environment in case of the isolates from the dam or might have been introduced as a growth promoter, prophylactically or for treatment of food animals that were slaughtered at the abattoir. The current study showed that all isolates from both sources were susceptible to colistin and tigecycline which are last line antibiotics. Colistin and tigecycline are normally used as a drug of last resort in hospitals which limits the possibility of bacteria developing resistance against these antibiotics. Also, they are rarely used in animals either as a growth promoter, prophylactically or for treatment. It is also noteworthy to highlight the fact that there was no statistical difference in the resistance rates of *A. baumannii* isolates recovered from the abattoir and from the aquatic environment (p < 0.253). This wide range of resistance, as demonstrated here, is a cause for concern.

In this study, isolates were also tested molecularly for gene similarities. Molecular analysis of the 48 and 52 MDR *A. baumannii* isolates showed that all isolates from both sources harboured $bla_{OXA-51-like}$ and 32 (66.7%) and 11 (21.2%) isolates harboured $bla_{OXA-23-like}$ genes in abattoir and aquatic isolates, respectively. This indicates that bla_{OXA-23} was the most prevalent carbapenem hydrolyzing class D β-lactamase gene frequently found in carbapenem-resistant *A. baumannii* isolates collected from extra-hospital environments. Our study showed that bla_{OXA-23} producers in particular and carbapenemase producers in general, may be isolated from extra-hospital environments. Among the hypotheses that could explain the selection of this carbapenemase is the indiscriminate use of penicillins or penicillin–β-lactamase inhibitor combinations. This could create selective pressure for β-lactamases because bla_{OXA-23} does confer, in addition to decreased susceptibility to carbapenems, a high level of

resistance to those compounds. It is well established that animals can be reservoirs of antimicrobial-resistant bacteria.[48] These identified determinants are not only of concern in *A. baumannii*. Rather, these genes can be transferred to other bacteria by horizontal gene transfer and are of great concern to human health. Therefore, there is the need to emphasize on strict adherence to personal and general hygiene to reduce the risk of opportunistic infection by *A. baumannii* which is difficult to control. Cases of *Acinetobacter* infection have been traced to environment due to poor hygiene, especially hand hygiene.[1]

The current study showed that all MDR isolates lacked significant antibiotic resistance features, such class 1 integrons and IS*Aba1*, indicating that the class 1 integron and IS*Aba1* associated resistance traits are not implicated in MDR of environmental *A. baumannii*. This is in agreement with a study conducted by Hamouda et al. where no class 1 integrons and IS*Aba1* were found in *A. baumannii* isolates from recently slaughtered animals destined for human consumption and collected at major Scottish abattoirs.[20] IS*Aab1* is considered the first step in resistance evolution in *A. baumannii*.[31] The absence of these resistance-driven mobile elements in these sources suggests that no or little antibiotic selective pressure was applied to these pathogens, contrary to their clinical counterpart. Therefore the high resistance rate to carbapenems which is the drug of choice to treat *A. baumannii* infections in both abattoir and aquatic isolates can be partly linked to the increased expression level of $bla_{OXA-51-like}$ carbapenemases. In addition, it is speculated that IS*Aba1* attains full expression under conditions of excessive selective pressure (presence of carbapenems); thus, one would not expect to find this mechanism of resistance in an environmental isolate.[14] However, three isolates (AB4, AB28 and AF3) that expressed $bla_{OXA-51-like}$ in our study unexpectedly showed imipenem MICs that were lower than 4 μg/mL, which suggests that imipenem resistance in some *A. baumannii* strains may be primarily modulated by genes other than $bla_{OXA-51-like}$. This can also be due to the $bla_{OXA-51-like}$ genes being usually silent or poorly expressed,[49] thus not conferring a resistance phenotype. Also, the increased use of carbapenems antibiotics to treat food producing animals may explain the high level of resistance to antibiotics of this class observed in the current study. The present results provide evidence that extra-hospital *A. baumannii* isolates serve as a reservoir of multiple antibiotics resistant and hence as potential route for the entry of MDR pathogens into human population. Our results also suggest that the nosocomial pathogen *A. baumannii* is well adapted to different environments, not only to the hospital setting. This has very important implications for human health, as infections by MDR are difficult to treat and often requires expensive antibiotics, long term therapy and even mortality. Further studies should be performed ascertain the prevalence of MDR *A. baumannii* in other sources.

Conclusion

This study has demonstrated by molecular identification that MDR *A. baumannii* is actually present outside the hospital. The

presence of MDR *A. baumannii* in the extra-hospital environment may be a threat to public health considering that this may provide a vector for the spread of these opportunistic pathogen into both community and hospital settings environment. Coordinated approaches to reduce integrated human health risks in the environment as well as careful compliance with the WHO guidelines[50] on surveillance, rational antibiotic prescribing, and standard treatment guidelines for both community- and hospital-acquired infections will lead appreciably towards reducing the ever-rising threat of antibiotic resistance. However, further studies are needed for better understanding of the mechanism of interactions between the different potential reservoirs and humans. Also, possible impact on the horizontal transfer of bla_{OXA} genes, surviving in selected condition or occurrence of infection outside the hospital setting should be further investigated.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article (and its supplementary information file).

Authors' contributions

Conceived and designed the experiments: S.S.P, T.A., S.D.V., A.Y.A. Performed the experiments: S.D.V., A.Y.A. Analysed the data: S.S.P, T.A., S.D.V, A.Y.A, O.G. E. Contributed reagents/materials/analysis tools: S.S.P, O.G. E., S.D.V Analysed data and wrote the manuscript: S.S.P, T.A., S.D.V, A.Y.A. All authors read and approved the final manuscript.

Ethical approval

Ethical approval for this project was obtained from the Walter Sisulu University Research Ethics Committee (Human) [Reference number: 019/2016].

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

REFERENCES

1. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev. 2008;21:538–82.
2. Eveillard M, Kempf M, Belmonte O, Pailhories H, Joly-Guillou ML. Reservoirs of *Acinetobacter baumannii* outside the hospital and potential involvement in emerging human community-acquired infections. Int J Infect Dis. 2013;17:e802–5.
3. Da Silva GJ, Domingues S. Insights on the horizontal gene transfer of carbapenemase determinants in the opportunistic pathogen *Acinetobacter baumannii*. Microorganisms. 2016;4: 29.
4. Higgins PG, Lehmann M, Wisplinghoff H, Seifert H. gyrB multiplex PCR to differentiate between *Acinetobacter calcoaceticus* and Acinetobacter genomic species 3. J Clin Microbiol. 2010;48:4592–4.
5. Belmonte O, Pailhories H, Kempf M, et al. High prevalence of closely-related *Acinetobacter baumannii* in pets according to a multicentre study in veterinary clinics, Reunion Island. Vet Microbiol. 2014;170:446–50 https://doi.org/10.1016/j.vetmic.2014.01.042
6. Stenström TA, Okoh AI, Adegoke AA. Antibiogram of environmental isolates of *Acinetobacter calcoaceticus* from Nkonkobe Municipality, South Africa. Fresenius Environ Bull. 2016;25:3059–65.
7. Fournier PE, Richet H. The epidemiology and control of *Acinetobacter baumannii* in health care facilities. Clin Infect Dis. 2006;42:692–9.
8. Ying C, Li Y, Wang Y, Zheng B, Yang C. Investigation of the molecular epidemiology of *Acinetobacter baumannii* isolated from patients and environmental contamination. J Antibiot (Tokyo). 2015;68:562–7.
9. Carvalheira A, Casquete R, Silva J, Teixeira P. Prevalence and antimicrobial susceptibility of *Acinetobacter* spp. isolated from meat. Int J Food Microbiol. 2017;243:58–63.
10. Naas T, Coignard B, Carbonne A, et al. VEB-1 extended-spectrum β-lactamase-producing *Acinetobacter baumannii,* France. Emerg Infect Dis. 2006;12:1214–22.
11. Rafei R, Hamze M, Pailhoriès H, et al. Extra-human epidemiology of *Acinetobacter baumannii* in Lebanon. Appl Environ Microbiol. 2015;81:2359–67.
12. Kempf M, Rolain JM. Emergence of resistance to carbapenems in *Acinetobacter baumannii* in Europe: clinical impact and therapeutic options. Int J Antimicrob Agents. 2012;39:105–14.
13. Kempf M, Rolain J-M, Diatta G, et al. Carbapenem resistance and *Acinetobacter baumannii* in Senegal: the paradigm of a common phenomenon in natural reservoirs. PLoS One. 2012;7:e39495 https://doi.org/10.1371/journal.pone.0039495
14. Carvalheira A, Ferreira V, Silva J, Teixeira P. Enrichment of *Acinetobacter* spp. from food samples. Food Microbiol. 2016;55:123–7.
15. Hrenović J, Durn G, Goić-Barišić I, Kovačić A. Occurrence of an environmental *Acinetobacter baumannii* strain similar to a clinical isolate in paleosol from Croatia. Appl Environ Microbiol. 2014;80:2860–6.
16. Choi J-Y, Kim Y, Ko EA, et al. *Acinetobacter* species isolates from a range of environments: species survey and

observations of antimicrobial resistance. Diagn Microbiol Infect Dis. 2012;74:177–80.

17. Dekić Svjetlana, Klobučar Göran, Ivanković Tomislav, et al. Emerging human pathogen *Acinetobacter baumannii* in the natural aquatic environment: a public health risk? Int J Environ Health Res. 2018;28:315–22, http://dx.doi.org/10.1080/09603123.2018.1472746.

18. Vaz-Moreira I, Nunes OC, Manaia CM. Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome. FEMS Microbiol. Rev. 2014;38:761–78.

19. Burger J. Consumption patterns and why people fish. Environ Res. 2002;90:125–35 (CAMS) Croatian Academy of Medical Sciences. 2017. Antibiotic resistance in Croatia, 2016. The Croatian Academy of Medical Sciences: Zagreb.

20. Hamouda A, Findlay J, Al Hassan L, Amyes SG. Epidemiology of *Acinetobacter baumannii* of animal origin. Int J Antimicrob Agents. 2011;38:314–8.

21. Poirel L, Berçot B, Millemann Y, Bonnin RA, Pannaux G, Nordmann P. Carbapenemase-producing *Acinetobacter* spp. in cattle, France. Emerg Infect Dis. 2012;18:523–5, http://dx.doi.org/10.3201/eid1803.111330.

22. Smet A, Boyen F, Pasmans F, et al. OXA-23-producing *Acinetobacter* species from horses: a public health hazard? J Antimicrob Chemother. 2012;67:3009–10.

23. Pomba C, Endimiani A, Rossano A, Saial D, Couto N, Perreten V. First report of OXA-23-mediated carbapenem resistance in sequence type 2 multidrug-resistant *Acinetobacter baumannii* associated with urinary tract infection in a cat. Antimicrob Agents Chemother. 2014;58:1267–8.

24. Wang Y, Wu C, Zhang Q, et al. Identification of New Delhi metallo-β-lactamase 1 in *Acinetobacter lwoffii* of food animal origin. PLoS One. 2012;7:e37152, http://dx.doi.org/10.1371/journal.pone.0037152.

25. Zhang WJ, Lu Z, Schwarz S, et al. Complete sequence of the *bla*(NDM-1)-carrying plasmid pNDM-AB from *Acinetobacter baumannii* of food animal origin. J Antimicrob Chemother. 2013;68:1681–2, http://dx.doi.org/10.1093/jac/dkt066.

26. Meat and Livestock Commission (MLC). International meat market review No. 26, MLC. Milton Keynes; 1999.

27. International Organization for Standardization (ISO). 17604. Microbiology of the food chain - Carcass sampling for microbiological analysis. Geneva: ISO; 2015.

28. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 27th ed Wayne, PA: CLSI; 2017. CLSI Document M100-27.

29. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 2012;18:268–81.

30. Krumperman PH. Multiple antibiotic resistance indexing of Escherichia coli to indentify high-risk sources of fecal contamination of foods. Applied Environ Microbiol. 1983;46:165–70.

31. Turton JF, Ward ME, Woodford N, et al. The role of ISAba1 in expression of OXA carbapenemase genes in Acinetobacter baumannii FEMS. Microbiol Lett. 2006;258:72–7.

32. Vanbroekhoven K, Ryngaert A, Wattiau P, De Mot R, Springael D. Acinetobacter diversity in environmental samples assessed by 16S rRNA gene PCR-DGGE fingerprinting. FEMS Microbiol Ecol. 2004;50:37–50.

33. Cherkaoui A, Emonet S, Renzi G, Schrenzel J. Characteristics of multidrug-resistant *Acinetobacter baumannii* strains isolated in Geneva during colonization or infection. Ann Clin Microbiol Antimicrob. 2015;14:1.

34. Da Silva GJ, Correia M, Vital C, et al. Molecular characterization of bla$_{IMP-5}$, a new integron-borne metallo-β-lactamase gene from an *Acinetobacter baumannii* nosocomial isolate in Portugal. FEMS Microbiol Lett. 2002;215:33–9.

35. Naas T, Ergani A, Carrër A, Nordmann P. Real-time PCR for detection of NDM-1 carbapenemase genes from spiked stool samples. Antimicrob Agents Chemother. 2011;55:4038–43.

36. Cardoso O, Leitao R, Figueiredo A, Sousa JC, Duarte A, Peixe LV. Metallo-beta-lactamase VIM-2 in clinical isolates of *Pseudomonas aeruginosa* from Portugal. Microb Drug Resist. 2002;8:93–7.

37. Héritier C, Poirel L, Nordmann P. Cephalosporinase over-expression resulting from insertion of ISAba1 in *Acinetobacter baumannii*. Clin Microbiol Infect. 2006;12:123–30.

38. Woodford N, Ellington MJ, Coelho JM, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents. 2006;27:351–3, http://dx.doi.org/10.1016/j.ijantimicag.2006.01.004.

39. Koeleman JGM, Stoof J, van der Bijl MW, Vandenbroucke-Grauls CMJE, Savelkoul PHM. Identification of epidemic strains of *Acinetobacter baumannii* by integrase gene PCR. J Clin Microbiol. 2001;39:8–13.

40. Gros M, PetrovicM, Barceló D. Multi-residue analytical methods using LC-tandem MS for the determination of pharmaceuticals in environmental and wastewater samples: a review. Anal Bioanal Chem. 2006;386:941–52.

41. British Society for Antimicrobial Chemotherapy, Version 14.0 Standing committee on susceptibility testing. Birmingham, UK: British Society for Antimicrobial Chemotherapy; 2015.

42. Francey T, Gaschen F, Nicolet J, Burnens AP. The role of *Acinetobacter baumannii* as a nosocomial pathogen for dogs and cats in an intensive care unit. J Vet Intern Med. 2000;14:177–83.

43. Chitanand MP, Kadam TA, Gyananath G, Totewad ND, Balhal DK. Multiple antibiotic resistance indexing of coliforms to identify high risk contamination sites in aquatic environment. Indian J microbiol. 2010;50:216–20.

44. Mak JK, Kim M-J, Pham J, Tapsall J, White PA. Antibiotic resistance determinants in nosocomial strains of multidrug-resistant *Acinetobacter baumannii*. J Antimicrob Chemother. 2009;63:47–54.

45. Riaz S, Faisal M, Hasnain S. Antibiotic susceptibility pattern and multiple antibiotic resistances (MAR) calculation of extended spectrum ß-lactamase (ESBL) producing *Escherichia coli* and Klebsiella species in Pakistan. Afr J Biotechnol. 2011;10:6325–31.

46. Suresh T, Srinivasan D, Hatha AAM, Lakshmanaperumalsamy P. The incidence, antibiotics resistance and survival of *Salmonella* and *Escherichia coli* isolated from broiler chicken retail outlets. Microbes Environ. 2000;15:173–81.

47. Gurung M, Nam HM, Tamang MD, et al. Prevalence and antimicrobial susceptibility of *Acinetobacter* from raw bulk tank milk in Korea. J Dairy Sci. 2013;96, http://dx.doi.org/10.3168/jds.2012-5965.

48. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. J Antimicrob Chemother. 2004;54:321–32.

49. Poirel L, Figueiredo S, Cattoir V, Carattoli A, Nordmann P. *Acinetobacter radioresistens* as a silent source of carbapenem resistance for Acinetobacter spp. Antimicrob Agents Chemother. 2008;52:1252–6.

50. World Health Organization. Step-by-step approach for development and implementation of hospital antibiotic policy and standard treatment guidelines 2011. Geneva, Switzerland: World Health Organization; 2011.