Microbiological Research 297 (2025) 128175

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

Outer membrane vesicles of carbapenem-resistant clinical *Acinetobacter baumannii* isolates protect both the vesicle-producing bacteria and non-resistant bacteria against carbapenems

Rodrigo Monteiro [a,b], Beatriz Santamarina Alcantud [a], Sjouke Piersma [a], Antoni P.A. Hendrickx [c], Sandra Maaß [d], Dörte Becher [d], Joana Azeredo [b], Erik Bathoorn [a], Jan Maarten van Dijl [a,*]

[a] University of Groningen, University Medical Center Groningen, Department of Medical Microbiology, Groningen, the Netherlands
[b] Centre of Biological Engineering, University of Minho, Campus de Gualtar, Braga, Portugal
[c] Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands
[d] University of Greifswald, Centre of Functional Genomics of Microbes, Department of Microbial Proteomics, Institute of Microbiology, Greifswald, Germany

ARTICLE INFO

Keywords:
Acinetobacter baumannii
Antibiotic-Resistance
Carbapenemases
Outer membrane vesicles
Virulence

ABSTRACT

Infections caused by carbapenem-resistant *Acinetobacter baumannii* (*A. baumannii*; CRAb) are associated with high patient morbidity and mortality. The serious threat for human health imposed by CRAb was recently underscored by identification of close-to-untouchable carbapenem- and tetracycline-resistant isolates. Since outer membrane vesicles (OMVs) of Gram-negative bacteria may contribute to antimicrobial resistance, our present study was aimed at investigating OMVs produced by the first two carbapenem- and tetracycline-resistant *A. baumannii* isolates in Europe. These isolates, denoted CRAb1 and CRAb2, contain large, nearly identical plasmids that specify multiple resistances. Both isolates produce OMVs that were analyzed by differential light scattering, transmission electron microscopy and proteomics. By comparison with OMVs from the plasmid-free non-carbapenem-resistant *A. baumannii* isolate Ab1, which is an isogenic ancestor of the CRAb1 isolate, we show that plasmid carriage by the CRAb1 and CRAb2 isolates leads to an increased OMV size that is accompanied by increased diversity of the OMV proteome. Our analyses show that OMVs from CRAb1 and CRAb2 are major reservoirs of proteins involved in antimicrobial resistance, including the plasmid-encoded carbapenemases New Delhi metallo-β-lactamase-1 (NDM-1), and carbapenem-hydrolyzing oxacillinase OXA-97 (OXA-97). Here we report that these OMV-borne carbapenemases hydrolyze imipenem and protect otherwise carbapenem-sensitive *A. baumannii* and *Escherichia coli* (*E. coli*) isolates against this antibiotic. In conclusion, our findings demonstrate that OMVs from highly drug-resistant CRAb confer protection against last-resort antibiotics to non-resistant bacterial pathogens.

1. Introduction

Acinetobacter baumannii (*A. baumannii*) is a Gram-negative bacterium that is notorious for causing life-threatening healthcare- and ventilator-associated pneumonia (Alrahmany et al., 2022; Du et al., 2019; Fitzpatrick et al., 2021; Pogue et al., 2022; Vivo et al., 2022). Particularly

Abbreviations: AR, antibiotic resistance; BCA, bicinchoninic; BLAST, basic local alignment search tool; CARD, comprehensive antibiotic resistance database; CFU, colony-forming units; CIM, carbapenem inactivation method; COG, cluster of orthologous genes; CRAb, carbapenem resistant *Acinetobacter baumannii*; DLS, dynamic light scattering; FDR, false discovery rate; H, hours; IBAQ, intensity-based absolute quantification; IMP, imipenem; LB, lysogeny broth; LC-MS/MS, liquid chromatography-mass spectrometry; LDS-PAGE, lithium dodecyl sulfate-polyacrylamide gel electrophoresis; MIC, minimum inhibitory concentration; Min, minutes; NDM-1, New Delhi metallo-β-lactamase-1; OD, optical density; OMV, outer membrane vesicle; OXA-97, carbapenem-hydrolyzing oxacillinase; PBS, phosphate-buffered saline; PDI, polydispersity index; PES, polyethersulfone; PMB, polymyxin B; PSM, peptide spectrum match; RI, refractory index; Rpm, revolutions per minute; S, seconds; SDS, Sodium dodecyl sulfate; SEM, standard error of the mean; SVM, support vector machine; TCA, trichloroacetic acid; TEM, transmission electron microscopy; WHO, world health organization.

* Correspondence to: University of Groningen, University Medical Center Groningen, Department of Medical Microbiology, Hanzeplein 1, P.O. Box 30001, Groningen 9700 RB, the Netherlands

E-mail address: j.m.van.dijl01@umcg.nl (J.M. van Dijl).

https://doi.org/10.1016/j.micres.2025.128175
Received 1 September 2024; Received in revised form 23 January 2025; Accepted 6 April 2025
Available online 12 April 2025

the emergence of carbapenem-resistant *A. baumannii* (CRAb) variants has raised the alarms due to their ability to resist last-generation β-lactam antibiotics. Combined with the already critical multidrug resistance in *A. baumannii*, the emergence of CRAb has increased patient morbidity and mortality due to *A. baumannii* infections even further (between 33 % and 86 %) (Benyamini, 2024; Du et al., 2019; Patel et al., 2019; Pogue et al., 2022). Furthermore, in the post-COVID-19 era, *A. baumannii* became one of the most frequently detected pathogens among patients in intensive care units (ICUs) of Chinese hospitals (20 %), where it especially affected patients with long stays (Yang et al., 2021). Also, other countries, like Spain, France, Japan, Egypt, Iran, and Italy, witnessed *A. baumannii* outbreaks among long-stay ICU patients who were treated for COVID-19 (Kyriakidis et al., 2021). These developments led the World Health Organization (WHO) to declare CRAb as a top-priority pathogen for the development of new antimicrobials in 2018, and this recommendation was reaffirmed by the WHO in 2024 (Tacconelli et al., 2018; WHO, 2024).

The main mechanisms underlying the rapid emergence of multidrug-resistant bacteria in general are: (i) horizontal transmission of antibiotic resistance genes via either transformation, transduction, or conjugation (J. Chen et al., 2018; McInnes et al., 2020; Muteeb et al., 2023; Thomas and Nielsen, 2005; Tran and Boedicker, 2017), and (ii) the mutation of specific genes that either lead to their overexpression or silencing, thereby causing increased antibiotic resistance (Revitt-Mills and Robinson, 2020; Stasiak et al., 2021). The antibiotic resistance mechanisms that have so far been identified in *A. baumannii* are based on enzymatic drug inactivation, limitation of drug uptake by changes in outer membrane permeability, drug target alterations as observed in penicillin-binding proteins, increased drug extrusion through overexpression of efflux pumps, and biofilm formation that establishes a physical barrier which protects the bacteria against antimicrobials (Shi et al., 2024). In the case of CRAb, resistance is mostly caused by the acquisition of plasmids that carry genes for carbapenemase enzymes, which hydrolyze carbapenems (Beig et al., 2023; Corvec et al., 2007). Tetracyclines are usually applied to treat CRAb infections but, unfortunately, tetracycline-resistant CRAb isolates have emerged (He et al., 2019; Isler et al., 2018). Such isolates can even be resistant to important last-line antibiotics, such as tigecycline, minocycline, eravacycline, and omadacycline (Chen et al., 2020; Evans et al., 2016; Livermore et al., 2016; Yang et al., 2022). The resistance to these antibiotics may be conferred by Tet(x) monooxygenase enzymes that can degrade tetracyclines (Chen et al., 2020; Yang et al., 2004).

Gram-negative bacteria like *A. baumannii* are known to discharge so-called outer membrane vesicles (OMVs) into their environment (Agarwal et al., 2019; Dhurve et al., 2022; Kesavan et al., 2020; Kwon et al., 2009; Li et al., 2015; Rumbo et al., 2011). OMVs can help the bacteria to thrive in different environments, providing a huge fitness advantage during the colonization of mammalian cells or surfaces by creating the first layers supporting biofilm formation (Baumgarten et al., 2012; Bielaszewska et al., 2018; Cañas et al., 2018; Tiku et al., 2021). Moreover, in addition to transformation, transduction and conjugation, these vesicles may be involved in horizontal gene transfer favoring the spread of antibiotic resistance genes, and they can help to disseminate quorum sensing molecules (Chatterjee et al., 2017; Mashburn and Whiteley, 2005; Rumbo et al., 2011). OMVs have also been implicated in bacterial virulence, for instance by neutralizing the host immune defenses (Espina et al., 2022; Gabarrini et al., 2018). Lastly, OMVs can provide different types of protection against environmental hazards, for instance by serving as decoys for bacteriophages (Manning and Kuehn, 2011), and by inactivating or binding antibiotics from different classes, like carbapenems (González et al., 2016; Kim et al., 2018; Lee et al., 2013) and polymyxins (Marchant et al., 2021; Park et al., 2021).

In the course of a recent *A. baumannii* outbreak in an ICU in the Netherlands, which initially started with carbapenemase-sensitive isolates, two tetracycline-resistant CRAb strains with distinct multi-locus sequence types were isolated from two of the affected patients (Talsma et al., 2024). Whole-genome sequencing showed that these isolates, denoted CRAb1 and CRAb2, carried close-to-identical plasmids of 265 kb with multiple antibiotic resistance genes, including the $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-97}}$ carbapenemase genes and the *tet(x3)* tetracycline resistance gene (Talsma et al., 2024) (Table 1). The *tet(x3)* gene was shown to confer high-level resistance to last-resort tetracyclines, including the newly registered eravacycline and omadacycline, which made these two isolates highly exceptional for CRAb isolates from Europe. In addition, the plasmids pCRAb1 and pCRAb2 had not been described before. Whole-genome sequencing of the first carbapenem-sensitive *A. baumannii* isolate (Ab1) from the outbreak's index patient showed that this isolate was isogenic with the CRAb1 isolate that was collected four weeks later from the same patient (Talsma et al., 2024). The Ab1 and CRAb1 isolates differed only for the presence of the 265-kb plasmid pCRAb1 in the CRAb1 isolate and the absence of a prophage from the CRAb1 isolate. Altogether, the collected data are indicative of inter-bacterial plasmid transfer during the outbreak, although the origin of the pCRAb1 and pCRAb2 plasmids remained unknown (Talsma et al., 2024).

Since previous studies have implicated OMVs in the Gram-negative bacterial resistance to antimicrobial agents, our present study was aimed at investigating whether the two tetracycline-resistant CRAb strains, CRAb1 and CRAb2, produce OMVs that contribute to these traits. To address this question, a proteomics approach was followed and combined with subsequent functional analyses. For comparison, we also analyzed the OMV proteome of the early non-resistant Ab1 isolate.

2. Materials and methods

2.1. Bacterial strains

The *A. baumannii* isolates used for this study were denoted Ab1 ($bla_{\text{ADC-79}}$, $bla_{\text{OXA-100}}$), CRAb1 ($bla_{\text{ADC-79}}$, $bla_{\text{OXA-100}}$, $bla_{\text{NDM-1}}$, $bla_{\text{OXA-97}}$, *tet(x3)*, *sul2*, *ant(2")-la*, *aph(3")-lb*, *aph(3')-la*, *aph(6)-ld*, *ant(3")-lla*, *mph(E)*, *msr(E)*), and CRAb2 ($bla_{\text{ADC-79}}$, $bla_{\text{OXA-100}}$, $bla_{\text{NDM-1}}$, $bla_{\text{OXA-97}}$, *tet(x3)*, *sul2*, *abaF*, *ant(2")-la*, *aph(3")-lb*, *aph(3')-la*, *aph(6)-ld*, *ant(3")-lla*, *mph(E)*, *msr(E)*). The genome sequences of these isolates have been submitted to the NCBI GenBank database and are available under the BioProject number PRJNA1011532. The isolates were routinely grown in Lysogeny Broth (LB, BD Difco™) at 37 °C. The cultures were shaken at 120 revolutions per minute (rpm) using a Multitron Triple Incubator Shaker (Infors HT). LB plates contained 1.2 % agar (BD Difco™). The cultures were routinely checked by plating on selective media.

2.2. Purification of OMVs

To isolate OMVs, overnight cultures of *A. baumannii* were used to inoculate 250 mL of LB at a 1:100 dilution. Subsequently, the bacteria were cultured for 24 h at 37 °C and 120 rpm. The bacteria were then

Table 1
Antibiotic resistance genes encoded by the large pCRAb1 and pCRAb2 plasmids in the CRAb1 and CRAb2 isolates.

Antibiotic resistance genes	Resistance against
$bla_{\text{ADC-79}}$	*β-lactams/carbapenems*
$bla_{\text{OXA-100}}$	
$bla_{\text{NDM-1}}$	
$bla_{\text{OXA-97}}$	
tet(x3)	Tetracyclines
sul2	Sulphonamides
ant(2")-la	Aminoglycosides
aph(3")-lb	
aph(3')-la	
aph(6)-ld	
ant(3")-lla	
mph(E)	Macrolides
msr(E)	

pelleted by centrifugation at 8000 × *g* for 20 minutes (min) at 4 °C, using an Avanti J-26S XP centrifuge (Beckman Coulter Inc., Brea, California, USA). The culture supernatant containing OMVs was filtered through a 0.22 μm polyethersulfone (PES) filter (VWR) and kept at 4 °C. OMVs were collected from the cell-free culture supernatant by ultracentrifugation at 30,000 rpm (150,000 × *g*) for 4 h, at 4 °C, using an OptimaTM XL-80K ultracentrifuge (Beckman Coulter Inc., Brea, California, USA). The supernatant was discarded, and the pellet was washed three times with phosphate-buffered saline (PBS) by ultracentrifugation at 100,000 × *g* for 3 h at 4 °C. OMVs were resuspended in 300–500 μL of PBS for further analysis. Quantification of the OMV protein content was performed using the Pierce™ bicinchoninic acid (BCA) Protein Assay Kit (ThermoFisher), and 2 % sodium dodecyl sulfate (SDS) for OMV solubilization (Morton and Evans, 1992).

2.3. Dynamic light scattering (DLS)

For DLS analysis, a Zetasizer Nano ZS (Malvern Panalytical Ltd, UK) was used. To this end, 40 μL of PBS-diluted OMVs (100–200 μg/mL) were submitted to DLS in micro cuvettes (part no. ZEN0040, Malvern Panalytical). The refractory index (RI) was set to 1.45 with an absorption of 0.001 and the dispersant RI was set to 1.335 with a viscosity of 0.8882. The temperature was set to 25 °C. Measurements were performed in triplicate with an equilibration time of 90 seconds (s).

2.4. Transmission electron microscopy (TEM)

For visualization of OMVs by TEM, negative staining was performed using a solution of 5 % uranyl acetate as previously described (Melo et al., 2014). A drop of the OMVs was deposited onto a copper grid with carbon-coated Formvar films. After 15 min of incubation, the grids were rinsed with ultrapure water, stained with uranyl acetate, and washed again before visualization using a Talos F200i electron microscope at 80 kV.

2.5. Lithium dodecyl sulfate (LDS) - polyacrylamide gel electrophoresis (PAGE)

OMVs samples (20 μg) were precipitated with trichloroacetic acid (TCA) at a final concentration of 10 % by incubation for 18 h at 4 °C. Subsequently, proteins were pelleted at 18,500 × *g*, at 4 °C for 20 min, washed with 1 mL of ice-cold acetone, centrifuged for 10 min, and dried by incubating the pellets at 60 °C for 10 min. Precipitated proteins were resuspended in a gel loading buffer containing NuPAGE 10 × sample reducing agent and 4 × LDS sample buffer (Invitrogen, USA), boiled at 98 °C for 10 min, and separated on 10 % NuPAGE gels (Invitrogen, USA) by LDS- polyacrylamide gel electrophoresis (PAGE). After electrophoresis proteins were stained with SimplyBlue SafeStain (Life Technologies, CA) and analyzed.

2.6. Liquid chromatography-mass spectrometry (LC-MS/MS) analyses

Gel lanes of LDS-PAGE gels with OMV samples (three independent biological replicates per strain) were excised in one piece and subjected to trypsin digestion as described earlier (Bonn et al., 2014). For the subsequent LC-MS/MS measurements, the digests were separated by reversed phase column chromatography using an EASY nLC 1000 (Thermo Fisher Scientific) with self-packed columns (OD 360 μm, ID 100 μm, length 20 cm) filled with 3 μm diameter C18 particles (Dr. Maisch, Ammerbuch-Entringen, Germany). Following loading/desalting in 0.1 % acetic acid in water, the peptides were separated by applying a binary non-linear gradient from 1 % to 99 % acetonitrile in 0.1 % acetic acid over 162 min. The LC was coupled online to a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher, Bremen, Germany) with a spray voltage of 2.6 kV. After a survey scan in the Orbitrap (r = 30,000), MS/MS data were recorded for the twenty most intensive precursor ions in the linear ion trap. Singly charged ions were not considered for MS/MS analysis. The lock mass option was enabled throughout all analyses.

After mass spectrometric measurement, a database search was performed using MaxQuant (version 2.0.3.0) with databases of *A. baumannii* Ab1, CRAb1, and CRAb2 isolates (3752, 3922, and 4119 entries, respectively) in separate (Cox and Mann, 2008). These databases were obtained from Prokka v1.14.6 annotation. For strain CRAb1 also protein sequences encoded by the plasmid (268 entries) were used for spectrum identification. Common laboratory contaminants and reversed sequences were included by MaxQuant. Search parameters were set as follows: trypsin/P specific digestion with up to two missed cleavages, methionine oxidation, and N-terminal acetylation as variable modification, match between runs with default parameters enabled. The false discovery rates (FDRs) of protein and the peptide spectrum match (PSM) levels were set to 0.01. Two identified unique peptides were required for protein identification. The values of accurate proteome-wide label free quantification by delayed normalization and maximal peptide ratio extraction (MaxLFQ) (Cox et al., 2014) and intensity-based absolute quantification (iBAQ) (Schwanhäusser et al., 2011) were calculated in MaxQuant with default settings as a proxy for protein abundance.

2.7. Analysis of proteome data

Functions of detected proteins were assessed using the 'basic local alignment search tool for proteins' (BLASTp) and manually curated using the 'universal protein knowledgebase' (UniProt) (Consortium, 2023). Cluster of orthologous genes (COG) categories were attributed using a public database of orthologous relationships, gene evolutionary history and functional annotations (eggNOG) (ver.6.0.0) (Huerta-Cepas et al., 2019), and reCOGnizer (ver.1.10.0) (Sequeira et al., 2022). The predicted subcellular localization of each identified OMV protein was assessed using an open-source tool for localization prediction in Gram-negative bacteria (PSORTb) (ver.3.0) (Yu et al., 2010), and literature data. For protein comparison across the isolates, Venn diagrams were drawn using InteractiVENN (Heberle et al., 2015). Antibiotic resistance proteins were predicted using the 'comprehensive antibiotic resistance database' (CARD) (Jia et al., 2017) as implemented in Abricate (Seemann, 2016). Virulence genes were determined by complementing the afore-mentioned tools with the VirulentPred algorithm (ver.1.0) (Garg and Gupta, 2008), which predicts bacterial virulence factors based on their amino acid composition, the bilayer cascade 'support vector machine' module (SVM), and the 'predict pathogenic proteins in metagenomic data sets' (MP3) webserver (Gupta et al., 2014).x

2.8. Assays for carbapenemase activity

Two procedures were applied to assay carbapenemase activity associated with OMVs: a biochemical chromogenic carbapenemase assay (CarbaNP test) (Bouslah, 2020) and the 'carbapenem inactivation method' (CIM) (van der Zwaluw et al., 2015). The CarbaNP test was adapted from a previous study (Pasteran et al., 2015) and was performed by mixing 100 μL containing 3.5 mg of OMVs with either solution A (100 μL of an aqueous solution of 0.05 % phenol-red with 10 mM of SO_4Zn), or solution B (solution A supplemented with imipenem at 6 mg/mL), followed by a 2 h incubation at 37 °C. Negative controls with or without imipenem, and with or without OMVs were used to accurately check for color changes. For the CIM analyses, a protocol was adapted from (van der Zwaluw et al., 2015). Briefly, an imipenem diffusion disk (BD BBL™ Sensi-Disc™, BD) was incubated for 2 h at 37 °C in 500 μL PBS with or without 2 mg/mL of OMVs. Afterwards, the disk was placed on an agar plate with a bacterial lawn of the imipenem susceptible indicator strain *A. baumannii* ATCC 17978. Positive controls were also prepared by incubating disks in PBS with the bacterial biomass

of each of the strains from which OMVs were derived. Agar plates were incubated overnight at 37 °C and the presence or absence of zones of growth inhibition was inspected.

2.9. Assay for OMV-mediated protection against imipenem

To evaluate the protection conferred by the OMVs against carbapenems, growth curves were recorded where the imipenem susceptible *A. baumannii* ATCC 17978 strain was incubated with antibiotics at a minimum inhibitory concentration (MIC), with or without CRAb2-derived OMVs. In particular, an overnight pre-inoculum of *A. baumannii* ATCC 17978 in LB was diluted to a cell density of 2×10^5 colony-forming units (CFUs)/mL and mixed with or without imipenem (0.125 μg/mL) and OMVs (20 μg) in a 96-well plate. The cultures were incubated in an Epoch plate reader (Biotek) for 6 h at 37 °C with continuous shaking (120 rpm) and with 30 min interval optical density measurements at 600 nm (OD_{600}). The experiment was performed in three independent biological replicates.

2.10. Assay for imipenem survival

Survival assays were performed by incubating *A. baumannii* ATCC 17978 and *E. coli* ATCC 25922, diluted from an overnight LB pre-inoculum to a cell density of 2×10^5 CFUs/mL, with an inhibitory antibiotic concentration (0.5 μg/mL of imipenem, 256 μg/mL of ampicillin) and three different CRAb2 OMV concentrations (10 μg/mL, 25 μg/mL, 50 μg/mL). The assay was carried out in 96-well plates that were incubated for 24 h at 37 °C without shaking. OD_{600} and CFUs were measured and compared to non-treated controls. The assay was performed in three different biological replicates.

2.11. Statistical analysis

Statistical analyses were performed using GraphPad software, version 7.0. All data are expressed as means ± standard error of the mean (SEM) and statistical differences were calculated by two-way ANOVA and Tukey's multiple comparison tests.

2.12. Data availability

All MS-based proteomics data have been deposited in the PRIDE repository of the ProteomeXchange Consortium (Deutsch et al., 2023) with the dataset identifier PXD051698.

3. Results and discussion

3.1. Characterization of OMVs

OMVs from the three *A. baumannii* study isolates Ab1, CRAb1 and CRAb2 were collected after 24 h culturing in LB. This involved subsequent centrifugation, ultracentrifugation and filtering steps after which the OMVs were resuspended in PBS. A first characterization of the OMVs was performed by DLS and TEM. The DLS measurements revealed that OMVs from all three isolates contained two differently sized populations. As presented in Table 2, two distinct peaks were detectable for each sample. OMVs from the Ab1 isolate had, on average, a smaller size than OMVs from the other two isolates. TEM images of the different OMV preparations showed that the smaller-sized population was prevalent, with very few OMVs of the higher-sized population (Fig. 1). The latter OMVs, together with some aggregated OMVs that were also detectable, could be responsible for the Peak 1 detected by DLS. These observations suggest that the actual size of OMVs is reflected by Peak 2 in the DLS measurements, which would be in accordance with the results from other studies (Agarwal et al., 2019; Kesavan et al., 2020; Li et al., 2015; Yun et al., 2018). Of note, Fig. 1C shows that phage particles adhere to some OMVs of the CRAb2 isolate which may, at least partially, explain the presence of Peak 1.

Table 2
DLS measurements on OMVs from the A. baumannii study isolates Ab1, CRAb1 and CRAb2.

A. baumannii isolates	Peak 1 Average size (nm)	Peak 2 Average size (nm)	PdI*
Ab1	194.9 ± 81.91	36.15 ± 9.61	0.309
CRAb1	275.5 ± 133.2	48.59 ± 14.89	0.494
CRAb2	266.1 ± 137.5	45.86 ± 13.04	0.409

*PdI refers to the polydispersity index, which indicates how homogeneous the respective sample is.

3.2. Proteomic analysis of OMVs

OMVs from the three study isolates were isolated and analyzed by MS for their protein content (Supplemental Table S1). The OMVs from the Ab1 strain presented the lowest protein diversity, with 162 different identified proteins (Fig. 2). The OMVs from the two CRAb isolates, CRAb1 and CRAb2, displayed significantly more protein diversity with, respectively, 406 and 522 identified proteins (Fig. 2). Since the Ab1 and CRAb1 strains are isogenic this suggests that the higher protein diversity in OMVs from the CRAb1 isolate is related to the large pCRAb1 plasmid that the CRAb1 isolate carries, or to the absence of a prophage from the CRAb1 isolate. However, since OMVs of the CRAb2 isolate show a comparably high protein diversity as OMVs from the CRAb1 isolate, it seems most likely that the elevated protein diversity of OMVs from the CRAb1 and CRAb2 isolates is due to the presence of pCRAb1 and pCRAb2, respectively.

As shown in Fig. 2 and Figure S1, the largest portion number of OMV proteins from the Ab1 isolate was predicted to be located in the outer membrane (33.33 %), whereas many other proteins were predicted to originate from multiple or unknown locations (19.14 %). The remaining identified OMV proteins from the Ab1 isolate were bioinformatically assigned to the inner membrane (12.96 %), cytoplasm (12.35 %), periplasm (11.73 %), or extracellular milieu (10.49 %). OMVs from the CRAb1 and CRAb2 isolates displayed a similar protein composition with nearly half of them having a predicted cytoplasmic localization (47.29 % and 57.28 %, respectively). This is a higher proportion of predicted cytoplasmic proteins than was observed for OMVs from the Ab1 isolate and could perhaps be related to the larger OMV sizes measured for the CRAb1 and CRAb2 OMVs. Both the CRAb1 and CRAb2 OMVs presented a lower proportion of predicted outer membrane proteins (13.55 % and 10.34 % respectively) compared to the Ab1 OMVs. Furthermore, a similar proportion of predicted inner membrane (15.76 % and 13.41 %), periplasmic (5.91 % and 4.41 %), and extracellular proteins (5.17 % and 2.68 %) was observed in the CRAb1 and CRAb2 OMVs, respectively. Lastly, a significant number of proteins with unknown localization (12.32 % and 11.88 %) was identified in OMVs from both CRAb isolates. Importantly, inspection of the total numbers of identified OMV proteins per predicted subcellular localization (Figure S1) shows that the main differences between OMVs from the Ab1 versus the CRAb1/CRAb2 proteins relate to proteins with a predicted cytoplasmic or inner membrane localization (Fig. 2). In contrast, comparable numbers of proteins with a predicted outer membrane, periplasmic, or extracellular localization were identified in OMVs of the three isolates.

To assess the similarities and differences in the identified protein content of OMVs from the three *A. baumannii* isolates, a BLASTp analysis was performed and the results are presented in Venn diagrams (Fig. 3). For this analysis, only proteins with an amino acid sequence with 100 % coverage and greater than 95 % identity were considered to be the same. The VENN diagram shows that, with 129 common proteins, there is a significant overlap in the OMV proteins from the three *A. baumannii* isolates. Yet, there are also clear differences. Specifically, 6 proteins are

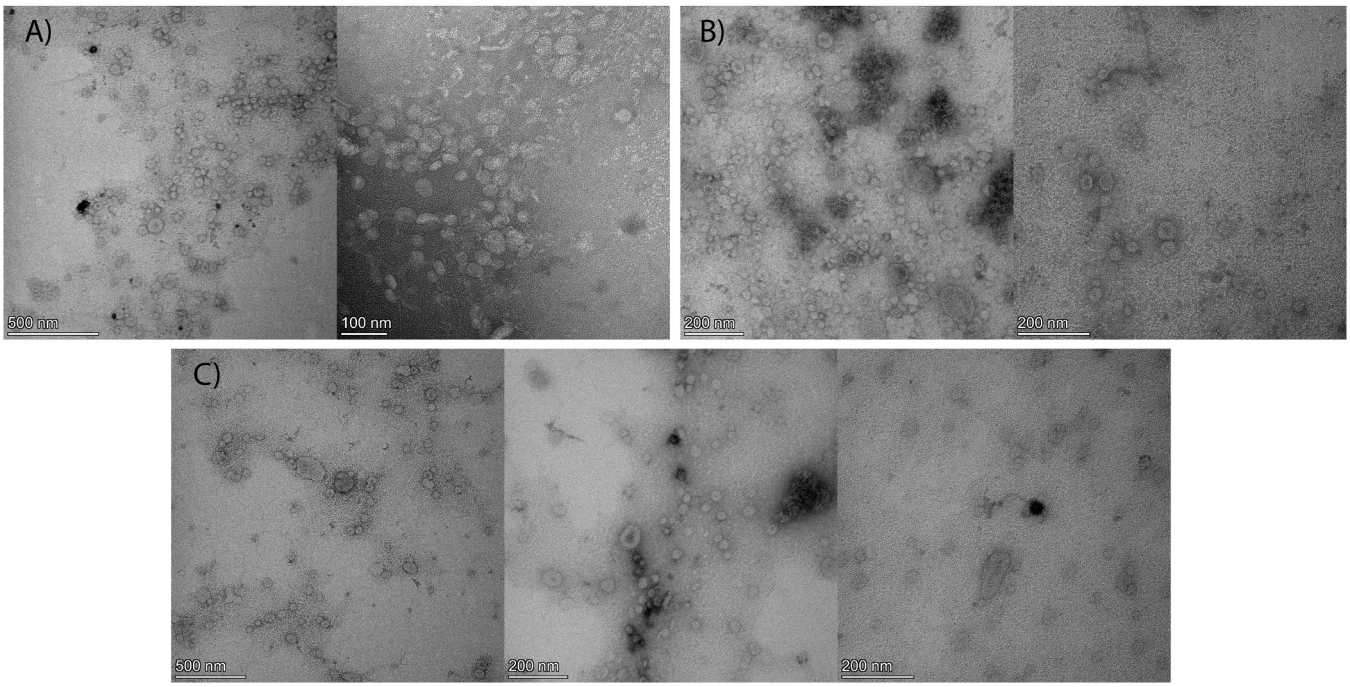

Fig. 1. Transmission electron miscroscopy (TEM) images of outer membrane vesicles (OMVs) from the three *A. baumannii* study isolates. A) Ab1; B) CRAb1; C) CRAb2.

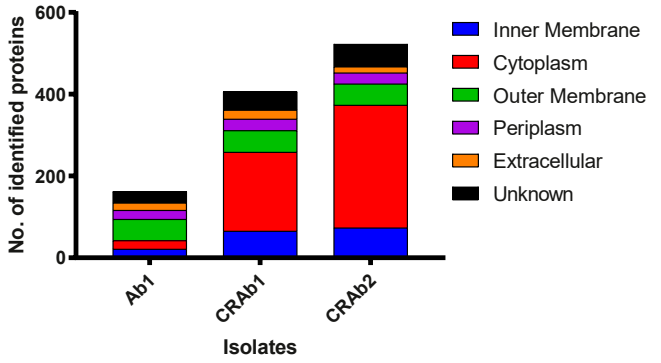

Fig. 2. Predicted localization of OMV-associated proteins from the Ab1, CRAb1 and CRAb2 isolates.

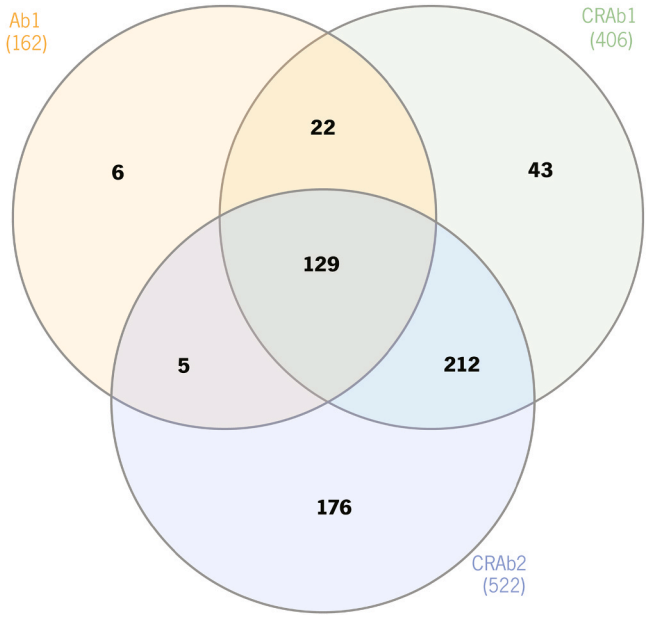

Fig. 3. Venn diagram showing the numbers of common and uniquely identified proteins in OMVs from the Ab1, CRAb1, and CRAb2 study isolates.

unique to Ab1 OMVs, 43 proteins to CRAb1 OMVs, and 176 proteins to CRAb2 OMVs. Despite the high genomic similarity between the Ab1 and CRAb1 isolates, there are still 11 OMV proteins from the Ab1 isolate that are not present in OMVs from the CRAb1 isolate, and 255 OMV proteins from the CRAb1 isolate that are absent from OMVs of the Ab1 isolate. None of the 11 unique proteins in Ab1 OMVs relate to the prophage that is absent from the CRAb1 isolate, which suggests that the carriage of plasmid pCRAb1 is largely responsible for the differences in the protein content of the Ab1 and CRAb1 OMVs. If so, the same is probably true for the presence of pCRAb2 in the CRAb2 isolate, which would imply that the plasmid carriage by the CRAb1 and CRAb2 isolates triggers the inclusion of additional cytoplasmic and inner membrane proteins in their OMVs. In this respect, it is noteworthy that in OMVs from the CRAb1 and CRAb2 isolates only four plasmid-encoded proteins were identified of which two were identified in OMVs from both isolates.

3.3. *OMV-borne proteins associated with antimicrobial resistance*

For functional annotation, proteins were analyzed using a combination of BLASTp, eggNOG, ABRicate, and manual curation based on UniProt. The analysis revealed that among the 129 common OMV-borne proteins, several were related to antibiotic resistance (AR), including the penicillin-binding protein 1B (Zapun et al., 2008), and the AdeK and AdeT RND-type efflux pumps (Damier-Piolle et al., 2008; Siroy et al., 2006). Notably, the carbapenemases OXA-97 and NDM-1, encoded by the $bla_{\text{OXA-97}}$ and $bla_{\text{NDM-1}}$ genes in the pCRAb1 and pCRAb2 plasmids, were present in the OMVs of the CRAb1 and CRAb2 isolates. Additionally, we found another β-lactamase, OXA-100, and the efflux pumps AdeJ and AdeI, which are chromosomally encoded and can confer resistance to a wide range of antibiotics (Damier-Piolle et al., 2008). Other proteins that potentially have a role in antimicrobial resistance,

such as the OmpW family outer membrane β-barrel protein (UniProt - ACIAD2272) (Roterman et al., 2021), the copper resistance protein B (Völlmecke et al., 2012), the CpaA metalloendopeptidase (Tilley et al., 2014), and the cobalt-zinc-cadmium resistance protein CzcA (Nies, 1995), were also identified. Table 3 lists all antibiotic-resistance proteins found across the OMVs of the different isolates. Each protein sequence was inspected by CARD (Jia et al., 2017). These observations show that *A. baumannii* OMVs can be regarded as reservoirs for proteins that confer antibiotic resistance, some of which may protect not only *A. baumannii* but also other bacterial cells against antimicrobials (Agarwal et al., 2019; Chatterjee et al., 2017; Rumbo et al., 2011).

3.4. OMV-associated carbapenemase activity

Previous studies have shown that different Gram-negative bacterial species, including *A. baumannii*, secrete β-lactamases in association with OMVs (Ciofu et al., 2000; González et al., 2016; Kim et al., 2018). Furthermore, (López et al., 2021) proposed that carbapenemases, such as NDM-1, are active in the periplasm, but can be packaged into OMVs due to electrostatic interaction with the outer membrane. Since we identified the carbapenemases OXA-97 and NDM-1 in OMVs from the CRAb1 and CRAb2 isolates, we investigated whether these enzymes are active and protect the bacteria against bactericidal carbapenem activity.

To assess OMV-associated carbapenemase activity, the CarbaNP test was performed (Pasteran et al., 2015). This test involved the mixing of OMVs with a phenol-red solution containing a carbapenem antibiotic. Since hydrolysis of the antibiotic by carbapenemase activity will lead to acidification of the solution, a color change from red to yellow is indicative of carbapenemase activity in the OMVs. Fig. 4 shows the color change from red to orange/yellow in samples that contained OMVs from the CRAb1 or CRAb2 isolates and imipenem, with a notable difference between the OMV-treated and the negative control samples. This result was further verified by recording the absorption spectra of the solutions, which clearly showed a shift from the red color peak (560 nm) to the yellow color peak (430 nm), with a red/yellow color ratio shift from 1.1 to 0.52 observe for the CRAb2 OMVs (Figure S2).

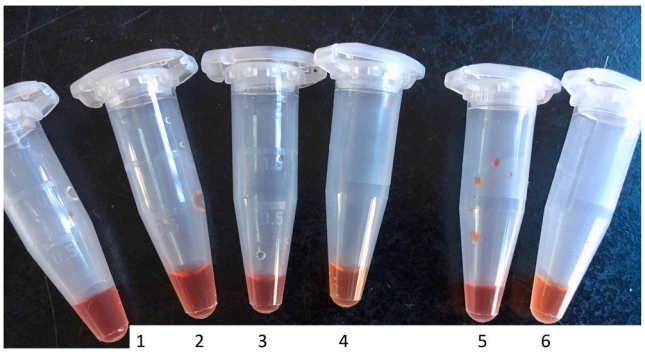

Fig. 4. Chromogenic carbapenemase (CarbaNP) test with isolated OMVs from the CRAb1 and CRAb2 isolates. Tubes from left to right show: 1 - negative control, phosphate-buffered saline (PBS); 2 - negative control, PBS with imipenem (IMP); 3 - CRAb1 OMVs, negative control without IMP; 4 - CRAb1 OMVs with IMP; 5 - CRAb2 OMVs, negative control without IMP; 6 - CRAb2 OMVs with IMP.

Table 3
Antimicrobial resistance proteins identified in the OMVs of the Ab1, CRAb1 and CRAb2 isolates.

Common in Ab1, CRAb1, and CRAb2 OMVs			
ID	Protein	Gene	CARD
P0003	AdeT, RND type efflux pump	*adeT*	-
P0009	Cobalt-zinc-cadmium resistance protein CzcA; efflux RND transporter permease subunit	*czcA*	*mexK*
P0169	Outer membrane protein OprM	*oprM*	*adeK*
P0222	Ornithine uptake porin CarO type 1	*carO*	*carO*
P0367	Cell envelope integrity protein TolA	*tolA*	-
P0478	Putative lipoprotein YiaD; OmpA-like	*yiaD*	*
P0522	Copper resistance protein B	*copB*	*
P0582	Outer membrane protein W	*ompW*	*
Common in Ab1 and CRAb1 OMVs			
ID	Protein	Gene	CARD
P0008	Macrolide export protein MacA	*macA*	*
P0523	Copper resistance protein A; copper resistance system multicopper oxidase	*copA*	-
P0265	OmpW family outer membrane ß-barrel protein	*omp*	*
Common in CRAb1 and CRAb2 OMVs			
ID	Protein	Gene	CARD
P0170	Multidrug resistance protein MexB; multidrug efflux RND transporter permease subunit AdeJ	*acrB*	*adeJ*
P0171	Multidrug efflux pump subunit AcrA	*acrA*	*adeI*
P0392	β-lactamase OXA−100	$bla_{OXA-100}$	OXA−100
P0644	β-lactamase OXA−97	bla_{OXA-97}	OXA−97
P0647	Metallo-β-lactamase type 2 NDM−1	bla_{NDM-1}	NDM−1
Unique to CRAb1 OMVs			
ID	Protein	Gene	CARD
P0062	Penicillin-binding protein 1 A	*mrcA*	*
Unique to CRAb2 OMVs			
ID	Protein	Gene	CARD
P0495	Colistin resistance protein EmrA; HlyD family secretion protein	*emrA*	*
P0532	Macrolide export protein MacA	*macA*	*

*CARD detected several homologous genes with low sequence identities (<60 %).

To test whether the OMV-associated carbapenemase activity could protect carbapenem-sensitive bacteria against such antibiotics, we applied the carbapenem inactivation method (CIM). This procedure is based on the incubation of a diffusion disk with imipenem in a solution containing OMVs or PBS, and the subsequent use of the incubated disk in a disk diffusion test on a plate with a susceptible strain. Lack of carbapenemase activity in the OMVs would not affect the antibiotic present in the disk and, accordingly, there would be a zone of growth inhibition detectable around the disk upon its usage in the disk diffusion test for carbapenem sensitivity. Conversely, OMV-associated carbapenemase activity would lead to diminished or no growth inhibition around the disk. Indeed, the results of the CIM showed that OMVs from the CRAb1 and CRAb2 isolates were capable of efficiently inactivating imipenem within the disc, while OMVs from the Ab1 isolate did not inactivate the imipenem in the disc (Fig. 5). As expected, the negative control of disk incubation without OMVs showed a clear zone of growth inhibition around the disk, while the positive controls of disk incubation with biomass of the CRAb1 or CRAb2 isolates in PBS resulted in the degradation of imipenem within the disk, as was evidenced by the absence of growth inhibition. Together, these results demonstrated the presence of active carbapenemases in the OMVs and implied that these OMVs can potentially contribute to increased bacterial tolerance to carbapenems.

3.5. OMV-mediated protection against imipenem

To assess the protection against carbapenems as conferred by the OMV-borne carbapenemases during bacterial growth, we incubated the carbapenem-susceptible strain *A. baumannii* ATCC 17978 with OMVs from the CRAb2 isolate in the presence or absence of imipenem. Ab1 OMVs were tested as well without any visible effect (data not shown). First, both OMVs (40 μg) and imipenem (0.125 μg/mL) were pre-incubated for 2 h at 37 °C before adding them to an equal volume of culture at an OD_{600} of 0.2 and, subsequently, growth was continued for 6 h. The results are shown in Fig. 6. During the first 30 min of the incubation, a decrease in the OD_{600} was observed, which was caused by the dilution of the culture upon mixing with the mixture of OMVs and imipenem. After 2 h of incubation, the growth rates started to diverge between cultures where imipenem was or was not pre-incubated with OMVs. After 4 h of incubation, the culture with OMV-pre-treated

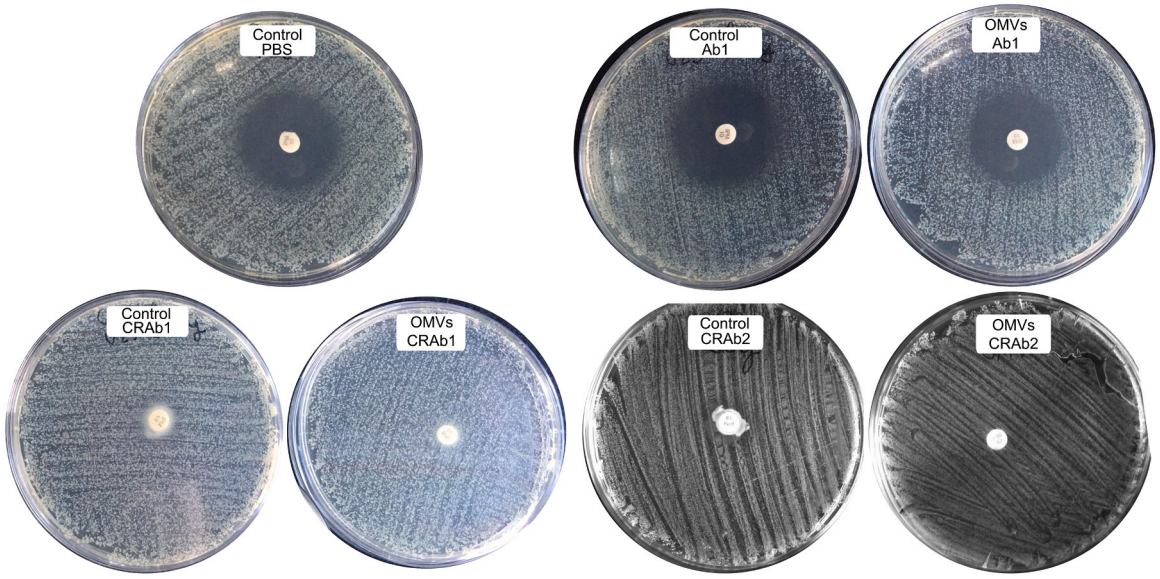

Fig. 5. Visualization of carbapenem inactivation by OMVs from the CRAb1 and CRAb2 study isolates using the 'carbapenemase inactivation method' (CIM) test. The carbapenem-susceptible strain used was *A. baumannii* 17978. Control phosphate-buffered saline (PBS), imipenem (IMP) diffusion disk incubated with PBS only. Control Ab1, CRAb1, and CRAb2, IMP diffusion disks incubated with biomass from the respective bacterial isolate resuspended in PBS. OMVs Ab1, CRAb1, and CRAb2, IMP diffusion disks incubated with OMVs from the respective bacterial isolate resuspended in PBS.

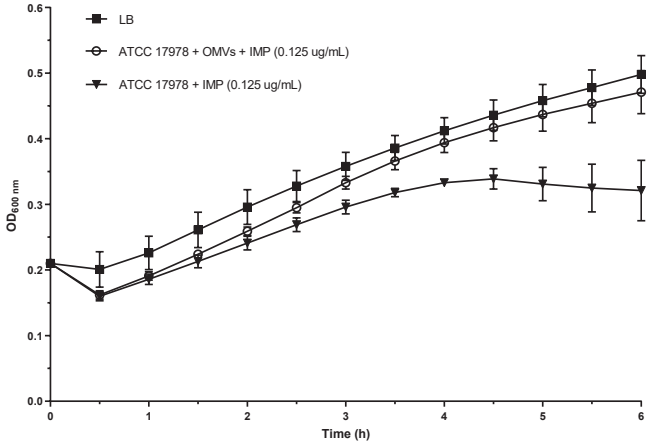

Fig. 6. Protection of the carbapenem-sensitive *A. baumannii* ATCC 17978 strain against growth inhibition by imipenem (IMP) upon IMP pretreatment with OMVs of the CRAb2 isolate. IMP (0.125 μg/mL) was mixed with OMVs (40 μg) or phosphate-buffered saline (PBS), and subsequently mixed with pre-cultured bacteria. Bacterial growth was measured by optical density readings at 600 nm for 6 h. As a control, pre-cultured bacteria without additions were mixed with lysogeny broth (LB).

imipenem continued to grow at the same rate as the LB control, whereas the culture with the non-pre-treated imipenem stopped growing and even showed a slightly decreased OD_{600}. This shows that the OMV-associated carbapenemase activity reduced the imipenem concentration to such an extent that the carbapenem-sensitive *A. baumannii* ATCC 17978 strain was able to grow. This observation is consistent with findings from other studies where β-lactamase-carrying OMVs from *E. coli* (Kim et al., 2018), *Staphylococcus aureus* (Lee et al., 2013), *Streptococcus pyogenes* (Bielaszewska et al., 2021), or *Stenotrophomonas maltophilia* (Devos et al., 2016) provided protection to β-lactam-susceptible strains of the same or other species. Furthermore, it was shown that OMVs of *A. baumannii* could protect a susceptible strain against polymyxin B (PMB) (Agarwal et al., 2019; Park et al., 2021). However, in the latter case, the OMVs most likely provided protection against PMB by binding this antibiotic and limiting the free PMB level rather than degrading it.

Lastly, we investigated the efficiency of the OMV-mediated protection against antibiotics by using different amounts of OMVs and checking for bacterial survival. For these experiments, we used two different antibiotic-susceptible species, namely *A. baumannii* ATCC 17978 and *E. coli* ATCC 25922. These bacteria were incubated with growth-inhibitory concentrations of imipenem, ampicillin, or ciprofloxacin, with or without three different concentrations of OMVs from the CRAb2 isolate (10/25/50 μg/mL). Of note, the MIC of ampicillin for the *A. baumannii* ATCC 17978 strain was very high and, therefore, only the *E. coli* ATCC 25922 strain was tested for OMV-medicated protection against this antibiotic. Cultures of *A. baumannii* ATCC 17978 and *E. coli* ATCC 25922 were incubated for 24 h and growth was measured by optical density readings and CFU counting. The results, in Fig. 7, showed that 10 μg/mL OMVs were enough to facilitate the survival of both bacterial species in the presence of imipenem, or the survival of *E. coli* ATCC 25922 in the presence of ampicillin. This result is consistent with the data of Kim and colleagues, who showed that OMVs from *E. coli* that carry β-lactamases can provide protection against ampicillin at an OMV concentration of at least 5 μg/mL (Kim et al., 2018). In the case of *A. baumannii* ATCC 17978, the bacterial growth in the presence of imipenem was fully restored at an OMV concentration of 25 μg/mL as was evident from optical density readings and CFU counting. Notably, a lower concentration of OMVs (10 μg/mL) already provided full protection against imipenem to *E. coli* ATCC 25922. This suggests that *E. coli* ATCC 25922 is more tolerant to imipenem than *A. baumannii* ATCC 17978. Similarly, when incubating the *E. coli* ATCC 25922 strain with OMVs and ampicillin, 25 μg/mL of OMVs was needed to fully restore growth to the level of the non-treated control. To verify that the observed OMV-medicated protection against the cell wall biogenesis-targeting antibiotics imipenem and ampicillin was specific, we used the fluoroquinolone antibiotic ciprofloxacin, which targets the topoisomerases II and IV in the bacterial cytoplasm. As shown in Fig. 7, there was no bacterial growth observed in cultures treated with OMVs and ciprofloxacin, which is consistent with the observation that no ciprofloxacin-resistance-related proteins were detected in the OMVs (Kulkarni et al., 2015; Marchant et al., 2021). Furthermore, we assessed whether the OMV-mediated protection against imipenem was perhaps

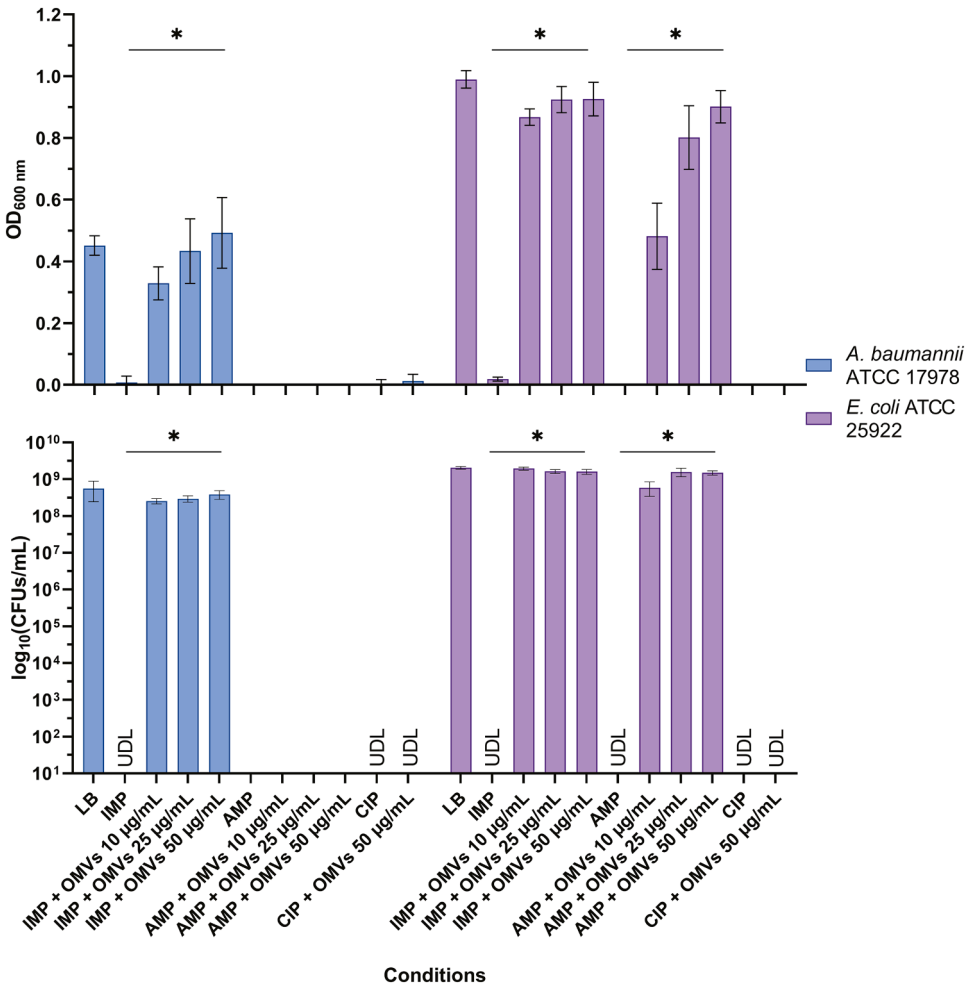

Fig. 7. *A. baumannii* ATCC 17978 and *E. coli* ATCC 25922 survival in the presence of imipenem, ampicillin, or ciprofloxacin facilitated by OMVs from the CRAb2 isolate. Bacterial growth was assayed by optical density readings (top) and colony-forming unit (CFU) counting (bottom). Lysogeny broth (LB), control without antibiotic nor OMVs; IMP, imipenem at 0.5 μg/mL; AMP, ampicillin at 256 μg/mL; CIP, Ciprofloxacin at 0.5 μg/mL. UDL, Under limit of detection. Asterisks indicate statistical significance for $p < 0.05$.

due to transfer of plasmid-borne resistance genes from the OMVs of the CRAb2 strain to *A. baumannii* ATCC 17978 or *E. coli* ATCC 25922, similar to what was previously reported for *E. coli* (Chatterjee et al., 2017; Rumbo et al., 2011; Yaron et al., 2000). However, upon plating the cultured bacteria on LB agar with imipenem, no growth of *A. baumannii* ATCC 17978 or *E. coli* ATCC 22975 was observed, showing that the OMVs mediated transient protection against imipenem.

4. Conclusion

The results of our present study show that two recently isolated highly drug-resistant clinical *A. baumannii* strains secrete OMVs loaded with different antibiotic resistance-related proteins. Among the OMV-borne antibiotic-resistance proteins, two carbapenemases were identified, which were highly active in the degradation of imipenem and provided protection against this antibiotic to other carbapenem-sensitive bacteria. The observed protection against imipenem was transient, meaning that there was no evidence for OMV-mediated carbapenemase gene transfer, despite the fact that several previous studies implicated OMVs from *A. baumannii* in the horizontal transfer of antibiotic resistance genes (Chatterjee et al., 2017; Rumbo et al., 2011). Altogether, our study highlights the possible role of OMVs in conferring protection against potent last-resort antibiotics to otherwise antibiotic-susceptible bacterial pathogens. Importantly, this mechanism of antibiotic resistance, which has been underappreciated for decades, may contribute to the emergence of *A. baumannii* and other opportunistic pathogens, lead to polymicrobial infections by dangerous pathogens, and increase the severity of bacterial infections by marginalizing the efficacy of antibiotic therapy (Weng et al., 2023). On the other hand, studies with OMVs have shown that they can potentially be applied for the development of alternative therapeutics, including vaccines for immunization against bacterial pathogens such as *A. baumannii* (Huang et al., 2014, 2016), or as vehicles for the delivery of drugs to sites of infection (Huang et al., 2020). In conclusion, OMVs of *A. baumannii* contribute to the antimicrobial resistance and virulence of this important pathogen, but they can potentially also be transformed into future drugs.

Funding

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, with DOI 10.54499/UIDB/04469/2020. RM acknowledges support from FCT doctoral fellowship, reference SFRH/BD/143639/2019.

CRediT authorship contribution statement

Bathoorn Erik: Writing – review & editing, Validation, Supervision, Resources, Methodology, Formal analysis, Conceptualization. **van Dijl**

Jan Maarten: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Becher Dörte:** Supervision, Resources, Project administration, Funding acquisition. **Azeredo Joana:** Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Hendrickx Antoni P.A.:** Writing – review & editing, Resources. **Maaß Sandra:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alcantud Beatriz Santamarina:** Investigation. **Piersma Sjouke:** Investigation. **Monteiro Rodrigo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

We thank Lilly Franzmeyer and Jürgen Bartel for technical support with the proteome analyses.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.micres.2025.128175.

Data availability

All MS data have been deposited in the PRIDE repository with the dataset identifier PXD051698.

References

Agarwal, B., Karthikeyan, R., Gayathri, P., RameshBabu, B., Ahmed, G., Jagannadham, M.V., 2019. Studies on the mechanism of multidrug resistance of Acinetobacter baumannii by proteomic analysis of the outer membrane vesicles of the bacterium. J. Proteins Proteom. 10 (1), 1–15. https://doi.org/10.1007/s42485-018-0001-4.

Alrahmany, D., Omar, A.F., Alreesi, A., Harb, G., Ghazi, I.M., 2022. Acinetobacter baumannii infection-related mortality in hospitalized patients: risk factors and potential targets for clinical and antimicrobial stewardship interventions. Antibiotics 11 (8). https://doi.org/10.3390/ANTIBIOTICS11081086.

Baumgarten, T., Sperling, S., Seifert, J., von Bergen, M., Steiniger, F., Wick, L.Y., Heipieper, H.J., 2012. Membrane Vesicle Formation as a Multiple-Stress Response Mechanism Enhances Pseudomonas putida DOT-T1E Cell Surface Hydrophobicity and Biofilm Formation. Appl. Environ. Microbiol. 78 (17), 6217–6224. https://doi.org/10.1128/AEM.01525-12.

Beig, M., Badmasti, F., Solgi, H., Nikbin, V.S., Sholeh, M., 2023. Carbapenemase genes distribution in clonal lineages of Acinetobacter baumannii: a comprehensive study on plasmids and chromosomes. Front. Cell. Infect. Microbiol. 13. https://doi.org/10.3389/fcimb.2023.1283583.

Benyamini, P., 2024. The comparative characterization of a hypervirulent acinetobacter baumannii bacteremia clinical isolate reveals a novel mechanism of pathogenesis. Int. J. Mol. Sci. 25 (18), 9780. https://doi.org/10.3390/ijms25189780.

Bielaszewska, M., Marejková, M., Bauwens, A., Kunsmann-Prokscha, L., Mellmann, A., Karch, H., 2018. Enterohemorrhagic Escherichia coli O157 outer membrane vesicles induce interleukin 8 production in human intestinal epithelial cells by signaling via Toll-like receptors TLR4 and TLR5 and activation of the nuclear factor NF-κB. Int. J. Med. Microbiol. 308 (7), 882–889. https://doi.org/10.1016/j.ijmm.2018.06.004.

Bielaszewska, M., Daniel, O., Nyč, O., Mellmann, A., 2021. In vivo secretion of β-lactamase-carrying outer membrane vesicles as a mechanism of β-lactam therapy failure. Membranes 11 (11). https://doi.org/10.3390/membranes11110806.

Bonn, F., Bartel, J., Büttner, K., Hecker, M., Otto, A., Becher, D., 2014. Picking Vanished Proteins from the Void: How to Collect and Ship/Share Extremely Dilute Proteins in a Reproducible and Highly Efficient Manner. Anal. Chem. 86 (15), 7421–7427. https://doi.org/10.1021/ac501189j.

Bouslah, Z., 2020. Carba NP test for the detection of carbapenemase-producing Pseudomonas aeruginosa. Médecine Et. Mal. Infect. 50 (6), 466–479. https://doi.org/10.1016/J.MEDMAL.2019.12.002.

Cañas, M.-A., Fábrega, M.-J., Giménez, R., Badia, J., Baldomà, L., 2018. Outer Membrane Vesicles From Probiotic and Commensal Escherichia coli Activate NOD1-Mediated Immune Responses in Intestinal Epithelial Cells. Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.00498.

Chatterjee, S., Mondal, A., Mitra, S., Basu, S., 2017. Acinetobacter baumannii transfers the blaNDM-1 gene via outer membrane vesicles. J. Antimicrob. Chemother. 72 (8), 2201–2207. https://doi.org/10.1093/jac/dkx131.

Chen, C., Cui, C.-Y., Yu, J.-J., He, Q., Wu, X.-T., He, Y.-Z., Cui, Z.-H., Li, C., Jia, Q.-L., Shen, X.-G., Sun, R.-Y., Wang, X.-R., Wang, M.-G., Tang, T., Zhang, Y., Liao, X.-P., Kreiswirth, B.N., Zhou, S.-D., Huang, B., Liu, Y.-H., 2020. Genetic diversity and characteristics of high-level tigecycline resistance Tet(X) in Acinetobacter species. Genome Med. 12 (1), 111. https://doi.org/10.1186/s13073-020-00807-5.

Chen, J., Quiles-Puchalt, N., Chiang, Y.N., Bacigalupe, R., Fillol-Salom, A., Chee, M.S.J., Fitzgerald, J.R., Penadés, J.R., 2018. Genome hypermobility by lateral transduction. Science 362 (6411), 207–212. https://doi.org/10.1126/science.aat5867.

Ciofu, O., Beveridge, T.J., Kadurugamuwa, J., Walther-Rasmussen, J., Høiby, N., 2000. Chromosomal beta-lactamase is packaged into membrane vesicles and secreted from Pseudomonas aeruginosa. J. Antimicrob. Chemother. 45 (1), 9–13. https://doi.org/10.1093/JAC/45.1.9.

Consortium, T.U., 2023. UniProt: the Universal Protein Knowledgebase in 2023. Nucleic Acids Res. 51 (D1), D523–D531. https://doi.org/10.1093/nar/gkac1052.

Corvec, S., Poirel, L., Naas, T., Drugeon, H., Nordmann, P., 2007. Genetics and Expression of the Carbapenem-Hydrolyzing Oxacillinase Gene blaOXA-23 in Acinetobacter baumannii. Antimicrob. Agents Chemother. 51 (4), 1530–1533. https://doi.org/10.1128/aac.01132-06.

Cox, J., Mann, M., 2008. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. Nat. Biotechnol. 26 (12), 1367–1372. https://doi.org/10.1038/nbt.1511.

Cox, J., Hein, M.Y., Luber, C.A., Paron, I., Nagaraj, N., Mann, M., 2014. Accurate Proteome-wide Label-free Quantification by Delayed Normalization and Maximal Peptide Ratio Extraction, Termed MaxLFQ. Mol. Cell. Proteom. 13, 2513–2526. https://doi.org/10.1074/mcp.

Damier-Piolle, L., Magnet, S., Brémont, S., Lambert, Thierry, Courvalin, P., 2008. AdeIJK, a Resistance-Nodulation-Cell Division Pump Effluxing Multiple Antibiotics in Acinetobacter baumannii. Antimicrob. Agents Chemother. 52 (2), 557–562. https://doi.org/10.1128/AAC.00732-07.

van der Zwaluw, K., de Haan, A., Pluister, G.N., Bootsma, H.J., de Neeling, A.J., Schouls, L.M., 2015. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. PloS One 10 (3), e0123690. https://doi.org/10.1371/journal.pone.0123690.

Deutsch, E.W., Bandeira, N., Perez-Riverol, Y., Sharma, V., Carver, J.J., Mendoza, L., Kundu, D.J., Wang, S., Bandla, C., Kamatchinathan, S., Hewapathirana, S., Pullman, B.S., Wertz, J., Sun, Z., Kawano, S., Okuda, S., Watanabe, Y., Maclean, B., Maccoss, M.J., Vizcaíno, J.A., 2023. The ProteomeXchange consortium at 10 years: 2023 update. Nucleic Acids Res. 51 (D1), D1539–D1548. https://doi.org/10.1093/NAR/GKAC1040.

Devos, S., Stremersch, S., Raemdonck, K., Braeckmans, K., Devreese, B., 2016. Intra-and interspecies effects of outer membrane vesicles from Stenotrophomonas maltophilia on β-lactam resistance. Antimicrob. Agents Chemother. 60 (4), 2516–2518. https://doi.org/10.1128/AAC.02171-15.

Dhurve, G., Madikonda, A.K., Jagannadham, M.V., Siddavattam, D., 2022. Outer Membrane Vesicles of Acinetobacter baumannii DS002 Are Selectively Enriched with TonB-Dependent Transporters and Play a Key Role in Iron Acquisition. Microbiol. Spectr. 10 (2). https://doi.org/10.1128/spectrum.00293-22.

Du, X., Xu, X., Yao, J., Deng, K., Chen, S., Shen, Z., Yang, L., Feng, G., 2019. Predictors of mortality in patients infected with carbapenem-resistant Acinetobacter baumannii: A systematic review and meta-analysis. Am. J. Infect. Control 47 (9), 1140–1145. https://doi.org/10.1016/j.ajic.2019.03.003.

Espina, M. du T., Fu, Y., van der Horst, D., Hirschfeld, C., López-Álvarez, M., Mulder, L. M., Gscheider, C., Rubio, A.H., Huitema, M., Becher, D., Heeringa, P., van Dijl, J.M., 2022. Coating and Corruption of Human Neutrophils by Bacterial Outer Membrane Vesicles. Microbiol. Spectr. 10 (5), e00753-22. https://doi.org/10.1128/spectrum.00753-22.

Evans, S.R., Hujer, A.M., Jiang, H., Hill, C.B., Hujer, K.M., Mediavilla, J.R., Manca, C., Tran, T.T.T., Nicholas, D.T., Higgins, P.G., Seifert, H., Kreiswirth, B.N., Patel, R., Jacobs, M.R., Chen, L., Sampath, R., Hall, T., Marzan, C., Fowler, V.G., Bonomo, R. A., 2016. Informing Antibiotic Treatment Decisions: Evaluating Rapid Molecular Diagnostics To Identify Susceptibility and Resistance to Carbapenems against Acinetobacter spp. in PRIMERS III. J. Clin. Microbiol. 55 (1), 134–144. https://doi.org/10.1128/jcm.01524-16.

Fitzpatrick, M.A., Suda, K.J., Poggensee, L., Vivo, A., Wirth, M., Wilson, G., Evans, M., Evans, C.T., 2021. Epidemiology and clinical outcomes associated with extensively drug-resistant (XDR) Acinetobacter in US Veterans' Affairs (VA) medical centers. Infect. Control Hosp. Epidemiol. 42 (3), 305–310. https://doi.org/10.1017/ICE.2020.450.

Gabarrini, G., Medina, L.M.P., Stobernack, T., Prins, R.C., Espina, M. du T., Kuipers, J., Chlebowicz, M.A., Rossen, J.W.A., van Winkelhoff, A.J., van Dijl, J.M., 2018. There's no place like OM: Vesicular sorting and secretion of the peptidylarginine deiminase of Porphyromonas gingivalis. Virulence 9 (1), 459–467. https://doi.org/10.1080/21505594.2017.1421827.

Garg, A., Gupta, D., 2008. VirulentPred: A SVM based prediction method for virulent proteins in bacterial pathogens. BMC Bioinforma. 9 (1), 1–12. https://doi.org/10.1186/1471-2105-9-62/FIGURES/5.

González, L.J., Bahr, G., Nakashige, T.G., Nolan, E.M., Bonomo, R.A., Vila, A.J., 2016. Membrane anchoring stabilizes and favors secretion of New Delhi metallo-β-lactamase. Nat. Chem. Biol. 12 (7), 516–522. https://doi.org/10.1038/nchembio.2083.

Gupta, A., Kapil, R., Dhakan, D.B., Sharma, V.K., 2014. MP3: A Software Tool for the Prediction of Pathogenic Proteins in Genomic and Metagenomic Data. PLOS ONE 9 (4), e93907. https://doi.org/10.1371/JOURNAL.PONE.0093907.

He, T., Wang, R., Liu, D., Walsh, T.R., Zhang, R., Lv, Y., Ke, Y., Ji, Q., Wei, R., Liu, Z., Shen, Y., Wang, G., Sun, L., Lei, L., Lv, Z., Li, Y., Pang, M., Wang, L., Sun, Q., Wang, Y., 2019. Emergence of plasmid-mediated high-level tigecycline resistance genes in animals and humans. Nat. Microbiol. 4 (9), 1450–1456. https://doi.org/10.1038/s41564-019-0445-2.

Heberle, H., Meirelles, V.G., da Silva, F.R., Telles, G.P., Minghim, R., 2015. InteractiVenn: A web-based tool for the analysis of sets through Venn diagrams. BMC Bioinforma. 16 (1), 1–7. https://doi.org/10.1186/S12859-015-0611-3.

Huang, W., Yao, Y., Long, Q., Yang, X., Sun, W., Liu, C., Jin, X., Li, Y., Chu, X., Chen, B., Ma, Y., 2014. Immunization against Multidrug-Resistant Acinetobacter baumannii Effectively Protects Mice in both Pneumonia and Sepsis Models. PLOS ONE 9 (6), e100727. https://doi.org/10.1371/JOURNAL.PONE.0100727.

Huang, W., Wang, S., Yao, Y., Xia, Y., Yang, X., Li, K., Sun, P., Liu, C., Sun, W., Bai, H., Chu, X., Li, Y., Ma, Y., 2016. Employing Escherichia coli-derived outer membrane vesicles as an antigen delivery platform elicits protective immunity against Acinetobacter baumannii infection. Sci. Rep. 2016 6 1 (6(1)), 1–12. https://doi.org/10.1038/srep37242.

Huang, W., Zhang, Q., Li, W., Yuan, M., Zhou, J., Hua, L., Chen, Y., Ye, C., Ma, Y., 2020. Development of novel nanoantibiotics using an outer membrane vesicle-based drug efflux mechanism. J. Control. Release 317, 1–22. https://doi.org/10.1016/J.JCONREL.2019.11.017.

Huerta-Cepas, J., Szklarczyk, D., Heller, D., Hernández-Plaza, A., Forslund, S.K., Cook, H., Mende, D.R., Letunic, I., Rattei, T., Jensen, L.J., von Mering, C., Bork, P., 2019. eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. Nucleic Acids Res. 47 (D1), D309–D314. https://doi.org/10.1093/nar/gky1085.

Isler, B., Doi, Y., Bonomo, R.A., Paterson, D.L., 2018. New Treatment Options against Carbapenem-Resistant Acinetobacter baumannii Infections, 10.1128/aac.01110-18 Antimicrob. Agents Chemother. 63 (1). https://doi.org/10.1128/aac.01110-18.

Jia, B., Raphenya, A.R., Alcock, B., Waglechner, N., Guo, P., Tsang, K.K., Lago, B.A., Dave, B.M., Pereira, S., Sharma, A.N., Doshi, S., Courtot, M., Lo, R., Williams, L.E., Frye, J.G., Elsayegh, T., Sardar, D., Westman, E.L., Pawlowski, A.C., McArthur, A.G., 2017. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res. 45 (D1), D566–D573. https://doi.org/10.1093/NAR/GKW1004.

Kesavan, D., Vasudevan, A., Wu, L., Chen, J., Su, Z., Wang, S., Xu, H., 2020. Integrative analysis of outer membrane vesicles proteomics and whole-cell transcriptome analysis of eravacycline induced Acinetobacter baumannii strains. BMC Microbiol. 20 (1). https://doi.org/10.1186/s12866-020-1722-1.

Kim, S.W., Park, S.Bin, Im, S.P., Lee, J.S., Jung, J.W., Gong, T.W., Lazarte, J.M.S., Kim, J., Seo, J.S., Kim, J.H., Song, J.W., Jung, H.S., Kim, G.J., Lee, Y.J., Lim, S.K., Jung, T.S., 2018. Outer membrane vesicles from β-lactam-resistant Escherichia coli enable the survival of β-lactam-susceptible E. coli in the presence of β-lactam antibiotics. Sci. Rep. 8 (1). https://doi.org/10.1038/s41598-018-23656-0.

Kulkarni, H.M., Nagaraj, R., Jagannadham, M.V., 2015. Protective role of E. coli outer membrane vesicles against antibiotics. Microbiol. Res. 181, 1–7. https://doi.org/10.1016/J.MICRES.2015.07.008.

Kwon, S.O., Gho, Y.S., Lee, J.C., Kim, S.Il, 2009. Proteome analysis of outer membrane vesicles from a clinical Acinetobacter baumannii isolate. FEMS Microbiol. Lett. 297 (2), 150–156. https://doi.org/10.1111/j.1574-6968.2009.01669.x.

Kyriakidis, I., Vasileiou, E., Pana, Z.D., Tragiannidis, A., 2021. Acinetobacter baumannii Antibiotic Resistance Mechanisms. Pathog. 2021, Vol. 10, Page 373 10 (3), 373. https://doi.org/10.3390/PATHOGENS10030373.

Lee, J., Lee, E.-Y., Kim, S.-H., Kim, D.-K., Park, K.-S., Kim, K.P., Kim, Y.-K., Roh, T.-Y., Gho, Y.S., 2013. Staphylococcus aureus extracellular vesicles carry biologically active β-lactamase. Antimicrob. Agents Chemother. 57 (6), 2589–2595. https://doi.org/10.1128/AAC.00522-12.

Li, Z.T., Zhang, R.L., Bi, X.G., Xu, L., Fan, M., Xie, D., Xian, Y., Wang, Y., Li, X.J., Wu, Z. D., Zhang, K.X., 2015. Outer membrane vesicles isolated from two clinical Acinetobacter baumannii strains exhibit different toxicity and proteome characteristics. Microb. Pathog. 81, 46–52. https://doi.org/10.1016/j.micpath.2015.03.009.

Livermore, D.M., Mushtaq, S., Warner, M., Woodford, N., 2016. In Vitro Activity of Eravacycline against Carbapenem-Resistant Enterobacteriaceae and Acinetobacter baumannii. Antimicrob. Agents Chemother. 60 (6), 3840–3844. https://doi.org/10.1128/aac.00436-16.

López, C., Prunotto, A., Bahr, G., Bonomo, R.A., González, L.J., Peraro, M.D., Vila, A.J., 2021. Specific Protein-Membrane Interactions Promote Packaging of Metallo-β-Lactamases into Outer Membrane Vesicles. Antimicrob. Agents Chemother. 65 (10). https://doi.org/10.1128/AAC.00507-21.

Manning, A.J., Kuehn, M.J., 2011. Contribution of bacterial outer membrane vesicles to innate bacterial defense. BMC Microbiol. 11. https://doi.org/10.1186/1471-2180-11-258.

Marchant, P., Carreño, A., Vivanco, E., Silva, A., Nevermann, J., Otero, C., Araya, E., Gil, F., Calderón, I.L., Fuentes, J.A., 2021. "One for All": Functional Transfer of OMV-Mediated Polymyxin B Resistance From Salmonella enterica sv. Typhi ΔtolR and ΔdegS to Susceptible Bacteria. Front. Microbiol. 12. https://doi.org/10.3389/FMICB.2021.672467/FULL.

Mashburn, L.M., Whiteley, M., 2005. Membrane vesicles traffic signals and facilitate group activities in a prokaryote. Nature 437 (7057), 422–425. https://doi.org/10.1038/nature03925.

McInnes, R.S., McCallum, G.E., Lamberte, L.E., van Schaik, W., 2020. Horizontal transfer of antibiotic resistance genes in the human gut microbiome. Curr. Opin. Microbiol. 53, 35–43. https://doi.org/10.1016/J.MIB.2020.02.002.

Melo, L.D.R., Sillankorva, S., Ackermann, H.W., Kropinski, A.M., Azeredo, J., Cerca, N., 2014. Isolation and characterization of a new Staphylococcus epidermidis broad-spectrum bacteriophage. J. Gen. Virol. 95 (PART 2), 506–515. https://doi.org/10.1099/vir.0.060590-0.

Morton, R.E., Evans, T.A., 1992. Modification of the bicinchoninic acid protein assay to eliminate lipid interference in determining lipoprotein protein content. Anal. Biochem. 204 (2), 332–334. https://doi.org/10.1016/0003-2697(92)90248-6.

Muteeb, G., Rehman, M.T., Shahwan, M., Aatif, M., 2023. Origin of Antibiotics and Antibiotic Resistance, and Their Impacts on Drug Development: A Narrative Review (Multidisciplinary Digital Publishing Institute (MDPI)). *Pharmaceuticals* 16 (11). https://doi.org/10.3390/ph16111615.

Nies, D.H., 1995. The Cobalt, Zinc, and Cadmium Efflux System CzcABC from Alcaligenes eutrophus Functions as a Cation-Proton Antiporter in Escherichia coli. J. Bacteriol. 177 (10), 2707–2712 (https://journals.asm.org/journal/jb).

Park, J., Kim, M., Shin, B., Kang, M., Yang, J., Lee, T.K., Park, W., 2021. A novel decoy strategy for polymyxin resistance in acinetobacter baumannii. ELife 10. https://doi.org/10.7554/eLife.66988.

Pasteran, F., Tijet, N., Melano, R.G., Corso, A., 2015. Simplified protocol for Carba NP test for enhanced detection of carbapenemase producers directly from bacterial cultures. J. Clin. Microbiol. 53 (12), 3908–3911. https://doi.org/10.1128/JCM.02032-15.

Patel, R.V., Shah, J.S., Revathi, G., Siika, W., Shah, R., 2019. Acinetobacter infections: a retrospective study to determine inhospital mortality rate and clinical factors associated with mortality. Infect. Prev. Pract. 1 (2), 100010. https://doi.org/10.1016/J.INFPIP.2019.100010.

Pogue, J.M., Zhou, Y., Kanakamedala, H., Cai, B., 2022. Burden of illness in carbapenem-resistant Acinetobacter baumannii infections in US hospitals between 2014 and 2019. BMC Infect. Dis. 22 (1). https://doi.org/10.1186/s12879-021-07024-4.

Revitt-Mills, S.A., Robinson, A., 2020. Antibiotic-Induced Mutagenesis: Under the Microscope (Frontiers Media S.A.). *Front. Microbiol.* 11. https://doi.org/10.3389/fmicb.2020.585175.

Roterman, I., Stapor, K., Fabian, P., Konieczny, L., 2021. The functional significance of hydrophobic residue distribution in bacterial beta-barrel transmembrane proteins. Membranes 11 (8). https://doi.org/10.3390/membranes11080580.

Rumbo, C., Fernández-Moreira, E., Merino, M., Poza, M., Mendez, J.A., Soares, N.C., Mosquera, A., Chaves, F., Bou, G., 2011. Horizontal transfer of the OXA-24 carbapenemase gene via outer membrane vesicles: A new mechanism of dissemination of carbapenem resistance genes in Acinetobacter baumannii. Antimicrob. Agents Chemother. 55 (7), 3084–3090. https://doi.org/10.1128/AAC.00929-10.

Schwanhäusser, B., Busse, D., Li, N., Dittmar, G., Schuchhardt, J., Wolf, J., Chen, W., Selbach, M., 2011. Global quantification of mammalian gene expression control. Nature 473 (7347), 337–342. https://doi.org/10.1038/nature10098.

Seemann, T. (2016). *ABRicate: mass screening of contigs for antibiotic resistance genes*. ⟨https://github.com/tseemann/abricate⟩ (0.9.8).

Sequeira, J.C., Rocha, M., Alves, M.M., Salvador, A.F., 2022. UPIMAPI, reCOGnizer and KEGGCharter: Bioinformatics tools for functional annotation and visualization of (meta)-omics datasets. Comput. Struct. Biotechnol. J. 20, 1798–1810. https://doi.org/10.1016/J.CSBJ.2022.03.042.

Shi, J., Cheng, J., Liu, S., Zhu, Y., Zhu, M., 2024. Acinetobacter baumannii: an evolving and cunning opponent (Frontiers Media SA). *Front. Microbiol.* 15. https://doi.org/10.3389/fmicb.2024.1332108.

Siroy, A., Cosette, P., Seyer, D., Lemaître-Guillier, C., Vallenet, D., Van Dorsselaer, A., Boyer-Mariotte, S., Jouenne, T., Dé, E., 2006. Global Comparison of the Membrane Subproteomes between a Multidrug-Resistant Acinetobacter baumannii Strain and a Reference Strain. J. Proteome Res. 5 (12), 3385–3398. https://doi.org/10.1021/pr060372s.

Stasiak, M., Maćkiw, E., Kowalska, J., Kucharek, K., Postupolski, J., 2021. Silent Genes: Antimicrobial Resistance and Antibiotic Production (Polish Society of Microbiologists). *Pol. J. Microbiol.* 70 (4). https://doi.org/10.33073/pjm-2021-040.

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D.L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., Outterson, K., Patel, J., Cavaleri, M., Cox, E.M., Houchens, C.R., Grayson, M.L., Hansen, P., Singh, N., Zorzet, A., 2018. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect. Dis. 18 (3), 318–327. https://doi.org/10.1016/S1473-3099(17)30753-3.

Talsma, D.T., Monteiro, R., Flores-Vallejo, R. del C., Heuvelmans, M., Le, T.-N., Hendrickx, A.P.A., Rosema, S., Maat, I., van Dijl, J.M., Bathoorn, E., 2024. Nosocomial transmission of tet(x3), blaNDM-1, and blaOXA-97-carrying Acinetobacter baumannii conferring resistance to eravacycline and omadacycline, the Netherlands, March to August 2021. Eur. Surveill. 29 (28). https://doi.org/10.2807/1560-7917.ES.2024.29.28.2400019.

Thomas, C.M., Nielsen, K.M., 2005. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat. Rev. Microbiol. 3 (9), 711–721. https://doi.org/10.1038/nrmicro1234.

Tiku, V., Kofoed, E.M., Yan, D., Kang, J., Xu, M., Reichelt, M., Dikic, I., Tan, M.-W., 2021. Outer membrane vesicles containing OmpA induce mitochondrial fragmentation to promote pathogenesis of Acinetobacter baumannii. Sci. Rep. 11 (1). https://doi.org/10.1038/s41598-020-79966-9.

Tilley, D., Law, R., Warren, S., Samis, J.A., Kumar, A., 2014. CpaA a novel protease from Acinetobacter baumannii clinical isolates deregulates blood coagulation. FEMS Microbiol. Lett. 356 (1), 53–61. https://doi.org/10.1111/1574-6968.12496.

Tran, F., Boedicker, J.Q., 2017. Genetic cargo and bacterial species set the rate of vesicle-mediated horizontal gene transfer. Sci. Rep. 7 (1), 8813. https://doi.org/10.1038/s41598-017-07447-7.

Vivo, A., Fitzpatrick, M.A., Suda, K.J., Jones, M.M., Perencevich, E.N., Rubin, M.A., Ramanathan, S., Wilson, G.M., Evans, M.E., Evans, C.T., 2022. Epidemiology and outcomes associated with carbapenem-resistant Acinetobacter baumannii and carbapenem-resistant Pseudomonas aeruginosa: a retrospective cohort study. BMC Infect. Dis. 22 (1), 491. https://doi.org/10.1186/s12879-022-07436-w.

Völlmecke, C., Drees, S.L., Reimann, J., Albers, S.-V., Lübben, M., 2012. The ATPases CopA and CopB both contribute to copper resistance of the thermoacidophilic archaeon Sulfolobus solfataricus. Microbiol. (Read., Engl.) 158 (Pt 6), 1622–1633. https://doi.org/10.1099/mic.0.055905-0.

Weng, Z., Yang, N., Shi, S., Xu, Z., Chen, Z., Liang, C., Zhang, X., Du, X., 2023. Outer Membrane Vesicles from Acinetobacter baumannii: Biogenesis, Functions, and Vaccine Application. Vaccines 12 (1), 49. https://doi.org/10.3390/vaccines12010049.

WHO, 2024. Bacterial Priority Pathogens List, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance, 2024. World Health Organization, Geneva.

Yang, J.-L., Yang, C.-J., Chuang, Y.-C., Sheng, W.-H., Chen, Y.-C., Chang, S.-C., 2022. Minocycline Susceptibility and tetB Gene in Carbapenem-Resistant Acinetobacter baumannii in Taiwan. Infect. Drug Resist. 15, 2401–2408. https://doi.org/10.2147/IDR.S357344.

Yang, S., Hua, M., Liu, X., Du, C., Pu, L., Xiang, P., Wang, L., Liu, J., 2021. Bacterial and fungal co-infections among COVID-19 patients in intensive care unit. Microbes Infect. 23 (4–5), 104806. https://doi.org/10.1016/j.micinf.2021.104806.

Yang, W., Moore, I.F., Koteva, K.P., Bareich, D.C., Hughes, D.W., Wright, G.D., 2004. TetX Is a Flavin-dependent Monooxygenase Conferring Resistance to Tetracycline Antibiotics. J. Biol. Chem. 279 (50), 52346–52352. https://doi.org/10.1074/jbc.M409573200.

Yaron, S., Kolling, G.L., Simon, L., Matthews, K.R., 2000. Vesicle-Mediated Transfer of Virulence Genes from Escherichia coli O157:H7 to Other Enteric Bacteria. Appl. Environ. Microbiol. 66 (10), 4414–4420 (https://journals.asm.org/journal/aem).

Yu, N.Y., Wagner, J.R., Laird, M.R., Melli, G., Rey, S., Lo, R., Dao, P., Cenk Sahinalp, S., Ester, M., Foster, L.J., Brinkman, F.S.L., 2010. PSORTb 3.0: improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. Bioinformatics 26 (13), 1608–1615. https://doi.org/10.1093/BIOINFORMATICS/BTQ249.

Yun, S.H., Park, E.C., Lee, S.-Y., Lee, H., Choi, C.-W., Yi, Y.-S., Ro, H.-J., Lee, J.C., Jun, S., Kim, H.-Y., Kim, G.-H., Kim, S.Il, 2018. Antibiotic treatment modulates protein components of cytotoxic outer membrane vesicles of multidrug-resistant clinical strain, Acinetobacter baumannii DU202. Clin. Proteom. 15 (1), 28. https://doi.org/10.1186/s12014-018-9204-2.

Zapun, A., Contreras-Martel, C., Vernet, T., 2008. Penicillin-binding proteins and beta-lactam resistance. FEMS Microbiol. Rev. 32 (2), 361–385. https://doi.org/10.1111/j.1574-6976.2007.00095.x.