UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
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DISSERTAÇÃO

GENÔMICA COMPARATIVA DE *Acinetobacter baumannii*: PAN-RESISTOMA E EVOLUÇÃO

DISCENTE: Diego Lucas Neres Rodrigues
ORIENTADOR: Prof. Dr. Vasco Ariston de Carvalho Azevedo
CO-ORIENTADORA: Dra. Flávia Figueira Aburjaile
CO-ORIENTADORA: Dra. Francielly Morais Rodrigues da Costa

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Diego Lucas Neres Rodrigues

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"Genômica Comparativa De Acinetobacter Baumannii: Pan-Resistoma e Evolução"

Diego Lucas Neres Rodrigues

Dissertação aprovada pela banca examinadora constituída pelos Professores:

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Dedico esse trabalho aos maiores incentivadores do meu desenvolvimento pessoal.

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“Continue a nadar.”

(Dori – Procurando Nemo, 2003)

RESUMO

Acinetobacter baumannii é um cocobacillo Gram-negativo que adquiriu um vasto repertório gênico de resistência à diversos antimicrobianos. Esse fato propiciou o protagonismo dessa bactéria em surtos epidemiológicos ao redor de todo o mundo. A Organização Mundial de Saúde (OMS) agrupa *A. baumannii* com outros microrganismos multirresistentes (*Multi Drug Resistant -MDR*), formando o grupo *ESKAPE* (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* e *Enterobacter spp.*), microrganismos de alta relevância clínica. Bactérias do gênero *Acinetobacter* também representam um grande problema para a Saúde Única (*One Health*) por também serem capazes de causar mastite em animais de produção, infecções severas em humanos e por vezes se apresentarem como contaminantes ambientais. Deste modo, para a reconstrução da resistência da espécie, foi necessário entender o desenvolvimento da problemática ao longo do tempo. Considerando a problemática exposta e a amplitude global de multirresistência e patogenicidade deste microrganismo, este trabalho tem como objetivo comparar *in silico* os genomas de 206 linhagens de *A. baumannii* com o intuito de realizar um estudo de metanálise da espécie e caracteriza-la por meio de análises de pangenoma e evolução. Como etapa inicial deste trabalho um estudo de metanálise foi desenvolvido para a prospecção literária de relatos clínicos de linhagens multi-resistentes de *A. baumannii* nas plataformas *NCBI* (*National Center for Biotechnology Information*) e *Cochrane*. Já para os estudos comparativos foram utilizados os dados genômicos de *A. baumannii* contra o banco de dados *Compreensive Antibiotic Resistance Database* (*CARD*) para a formação do resistoma da espécie. Em seguida foi analisada a distribuição filogenética das espécies dentro do gênero com base em genes *housekeeping* e a reconstrução filogenômica da espécie *A. baumannii* com base em genes e proteínas pertencentes ao genoma central. As linhagens bacterianas foram separadas com base em Sequência Tipo e Identidade Média de Nucleotídeos (*Average Nucleotide Identity - ANI*). Posteriormente, foi realizada a análise da plasticidade genômica utilizando o software *Genomic Island Prediction Software* (*GIPSy*) e a análise do repertório total genético da espécie utilizando o *software OrthoFinder*. Com isso, os principais resultados obtidos foram: (i) basendo em Odds Ratio, *A. baumannii* possui cerca de 355% a chance de resistência à carbapenem quando comparada à chance de outras bactérias; (ii) o repertorio de genes de resistência da espécie é formado por 171 genes; (iii) há uma maior proporção de genes relacionados à resistência contra β-lactâmicos no geral, aminoglicosídeos, tetraciclinas; (iv) a quantidade de genes de resistência à gliciclina e às polimixinas encontrada foi inferior à das demais classes relevantes contra a espécie, como β-lactâmicos; (v) a distribuição global das linhagens de *A. baumannii* é homogênea e apresenta baixa clonalidade por isolamento geográfico considerando as Sequencias Tipo da espécie; (vi) o pangenoma da espécie é aberto, tendo um genoma central formado por 1.999 genes, e um genoma acessório formado por 12.336 genes. Portanto, conclui-se que o repertório de genes de resistência da espécie é vasto e complexo, além de condizer com os diversos casos clínicos relatados, o que indica que sua presença é crucial para a resistência bacteriana e seu sucesso como patógeno. Com isso, foi possível sumarizar os resultados de diferentes épocas e compreender melhor a origem e evolução da resistência da espécie. Análises futuras podem ser elaboradas para comparar os resultados aqui apresentados com outros microrganismos igualmente problemáticos. Além disso, é possível aplicar os resultados encontrados na análise pangenômica para a prospecção de candidatos vacinais e alvos terapêuticos contra infeções por *A. baumannii*.

Palavras-chave: Bactérias multirresistentes, metanálise, resistência bacteriana, pangenoma, patogenômica.

ABSTRACT

Acinetobacter baumannii is a Gram-negative coccobacillus that has acquired a vast resistance gene repertoire against several antimicrobials. This fact led to the prominence of this bacterium in epidemiological outbreaks around the world. The World Health Organization (WHO) groups *A. baumannii* with other Multi-Drug Resistant (MDR) microorganisms, forming the ESKAPE group of clinical relevance with *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter spp*. Bacteria of the genus *Acinetobacter* also represent a significant problem for One Health because they are also capable of causing mastitis in farm animals, severe infections in humans, and sometimes are identified as environmental contaminants. Thus, for the reconstruction of the species' resistance, it was necessary to understand the development of the problem over time. This work aims to compare *in silico* the genomes of 206 *A. baumannii* strains to carry out a study of the meta-analysis of the species and characterize it through analysis of pan-genome and evolution Considering the exposed problem and the global amplitude of multidrug resistance and pathogenicity of this microorganism. As an initial stage of this work, a meta-analysis study was developed for the literary prospection of clinical reports of multi-resistant strains of *A. baumannii* on the NCBI (National Center for Biotechnology Information) and Cochrane platforms. For the comparative studies, the genomic data of *A. baumannii* was used against the Comprehensive Antibiotic Resistance Database (CARD) for the formation of the species' resistome. Then, the phylogenetic distribution of the species within the genus was analyzed based on housekeeping genes and the phylogenomic reconstruction of the species *A. baumannii* based on genes and proteins belonging to the central genome. The bacterial strains were separated based on Type Nucleotide Sequence and Average Nucleotide Identity (ANI). Subsequently, the genomic plasticity analysis was performed using the Genomic Island Prediction Software (GIPSy) and the analysis of the total genetic repertoire of the species using the OrthoFinder software. Thus, the main results obtained were: (i) based on Odds Ratio, *A. baumannii* has about 355% chance of resistance to carbapenem when compared to the chance of other bacteria; (ii) the repertoire of resistance genes of the species is formed by 171 genes; (iii) there is a higher proportion of genes related to resistance against β-lactams in general, aminoglycosides, tetracyclines; (iv) the amount of glycine and polymyxin resistance genes found was lower than that of the other relevant classes against the species, such as β-lactams; (v) the global distribution of *A. baumannii* strains is homogeneous and has low clonality due to geographic isolation considering the Type Sequences of the species; (vi) the pan-genome of the species is open, having a central genome formed by 1,999 genes, and an accessory genome formed by 12,336 genes. Therefore, it is concluded that the repertoire of resistance genes of the species is vast and complex, in addition to matching the various clinical cases reported, which indicates that its presence is crucial for bacterial resistance and its success as a pathogen. With that, it was possible to summarize the results of different times and better understand the origin of the evolution of the species' resistance. Future analyses can be carried out to compare the results presented here with other equally problematic microorganisms. In addition, it is possible to apply the results found in the pangenomic analysis to search for vaccine candidates and therapeutic targets against *A. baumannii* infections.

Keywords: Multidrug-resistant bacteria, meta-analysis, bacterial resistance, pan-genome, pathogenomics**.**

LISTA DE ABREVIATURAS

ANI	Average Nucleotide Identity
CARD	Comprehensive Antibiotic Resistance Database
GIPSy	Genomic Island Prediction Software
KEGG	Kyoto Encyclopedia of Genes and Genomes
LPSN	List of Prokaryotic names with Standing in Nomenclature
LGCM	Laboratório de Genética Celular e Molecular
MDR	Multidrug Resistant
MLST	Multi-Locus Sequence Typing
NCBI	National Center for Biotechnology Information
OMS	Organização Mundial da Saúde
ST	Sequência Tipo
WHO	World Health Organization

SUMÁRIO

1. APRESENTAÇÃO

Colaboradores e Financiamento

O seguinte trabalho foi desenvolvido no Laboratório de Genética Celular e Molecular (LGCM), coordenado pelo pesquisador responsável Prof. Dr. Vasco Azevedo e pertencente ao Departamento de Genética, Ecologia e Evolução do Instituto de Ciências Biológicas (ICB) na Universidade Federal de Minas Gerais (UFMG). Esta dissertação foi supervisionada pelo Prof. Dr. Vasco Azevedo, Dra. Flávia Aburjaile e Dra. Francielly Morais Rodrigues da Costa.

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2. INTRODUÇÃO

2.1. Origem, evolução e biologia de *Acinetobater baumannii*

O gênero *Acinetobacter* pertence à família *Moraxellaceae* e à ordem *Pseudomonadales* (YANG, 2014). Tratam-se de bactérias Gram-negativas, oxidase-negativas, catalase-positivas e não-fermentadoras (PELEG; SEIFERT; PATERSON, 2008). Bactérias pertencentes a esse gênero possuem em média um conteúdo GC de 39.6% e 3.87Mb de acordo com genomas depositados no banco de dados do *National Center for Biotechnology Information* (*NCBI*) (TOUCHON *et al.*, 2014). Atualmente esse táxon engloba 65 espécies publicadas, segundo a curadoria realizada pelo projeto *List of Prokaryotic names with Standing in Nomenclature* (*LPSN*) (PARTE *et al.*, 2020). Dentro do táxon, o organismo de maior importância é o *A. baumannii* devido à sua alta resistência, capacidade de sobrevivência em ambientes pouco comuns e formação de biofilme (HOWARD *et al.*, 2012).

Evolutivamente, uma das principais hipóteses levantadas quanto ao evento de especiação de *A. baumannii* é baseada na teoria de que sua origem se deu inicialmente em um estado de súbita redução populacional em um processo conhecido como efeito gargalo (TOUCHON *et al.*, 2014). Sabe-se que esse evento tende a promover a especiação de uma população a custo de grande redução da variabilidade genética (CHAKRABORTY; KIMMEL, 2001). Contudo, o conteúdo genômico de *A. baumannii* é extremamente diverso e plástico (PELEG; SEIFERT; PATERSON, 2008). Sugere-se, portanto, que após o efeito gargalo houve uma massiva expansão populacional mediada por um processo de seleção purificadora que possivelmente eliminou as mutações não-sinônimas do material genético da espécie (TOUCHON *et al.*, 2014).

Estudos apontam que o *A. baumannii* se tornou um problema clínico relevante em meados da década de 70 (BERGOGNE-BÉRÉZIN, 1994). Os principais acometimentos desse microrganismo foram: bacteremia (ELIOPOULOS; MARAGAKIS; PERL, 2008), infecção do trato respiratório superior e pneumonia (ANTUNES; VISCA; TOWNER, 2014; JUNG *et al.*, 2017); cistite (JIMÉNEZ-GUERRA *et al.*, 2018); infecção de partes moles e osteomielite (VANEGAS *et al.*, 2015); meningite (SIEGMAN-IGRA *et al.*, 1993); e conjuntivite (MCGRATH *et al.*, 2011). Sendo inclusive um problema relacionado ao conceito de saúde única ao causar mastite, bacteremia e pneumonia também em animais de produção (WARETH *et al.*, 2019).

A espécie não é exclusivamente um patógeno intracelular, porém é capaz de infectar, persistir e se reproduzir no interior de células eucarióticas podendo induzir apoptose, em especial em macrófagos e células epiteliais (CHOI *et al.*, 2005; QIN *et al.*, 2020). Um estudo conduzido em 2020 sugere que células bacterianas de *A. baumannii* podem se manter presentes no interior de vacúolos autofágicos, no entanto, evitam se manter próximas de compartimentos lisossomais tendendo a manter menor distância das estruturas dos retículos endoplasmáticos (QIN *et al.*, 2020). Esse fato se relaciona diretamente com a subversão do sistema imune (ROY; SALCEDO; GORVEL, 2006).

A epidemiologia da espécie é difusa. É um microrganismo cosmopolita democrático que já causou e ainda causa surtos epidêmicos ao redor do mundo. Já foram relatados casos em países como África do Sul (SNYMAN *et al.*, 2020), Brasil e Argentina (RODRÍGUEZ; NASTRO; FAMIGLIETTI, 2018), Arábia Saudita (IBRAHIM, 2019), Estados Unidos (PELEG; SEIFERT; PATERSON, 2008), França (POTRON; POIREL; NORDMANN, 2015), Itália (MEZZATESTA *et al.*, 2012), entre outros, como pode ser visto em outros trabalhos (PELEG; SEIFERT; PATERSON, 2008).

Muito autores consideram *A. baumannii* uma bactéria ubíqua devido ao seu gênero (HOWARD *et al.*, 2012), porém o seu grau de especialização a permite ser encontrada com menor frequência em amostras ambientais e sua ubiquidade é atualmente questionável (ANANE A *et al.*, 2019). *A. baumannii* é também comumente encontrada na microbiota de transição da pele e trato respiratório superior, geralmente provocando um estado de doença apenas em caso de distúrbio da homeostasia (FOURNIER, Pierre Edouard; RICHET; WEINSTEIN, 2006).

O diagnóstico clínico de infecções por *A. baumannii* pode se dar por meio de ensaios bioquímicos e moleculares. Existem hoje mais de 28 testes bioquímicos para identificar as bactérias do gênero, porém esses ensaios podem não revelar a identificação da espécie (HOWARD *et al.*, 2012; PELEG; SEIFERT; PATERSON, 2008). Para a uma caracterização mais específica, ensaios moleculares específicos são realizados pela busca de marcadores, sendo genes *housekeeping* (como o 16S rRNA) ou *fingerprints* em regiões espaçadoras (HOWARD *et al.*, 2012). Além disso, uma outra técnica difundida, é a tipagem molecular ou tipagem de sequência multilocus (*Multi-Locus Sequence Typing – MLST*), muito empregada na diferenciação de linhagens ou espécies bacterianas. É uma ferramenta já consolidada no meio acadêmico e clínico, fazendo parte do repertório de técnicas de classificação taxonômica de rotina (MAIDEN, 2006; MAIDEN *et al.*, 1998). Nos últimos anos, dois grupos de pesquisa publicaram esquemas para a tipagem molecular de *A. baumannii* baseados em variantes de um

conjunto de sete genes *housekeeping*, sendo ambos eficientes na diferenciação da espécie quando o sequenciamento do genoma está disponível (BARTUAL *et al.*, 2005; DIANCOURT *et al.*, 2010).

2.2. Resistência microbiana da espécie

Recentemente, avaliações dos motivos de resistência a antimicrobianos ligados à genética de diferentes linhagens da espécie tem sido divulgados (ASIF; ALVI; REHMAN, 2018; JAIDANE *et al.*, 2018; LEE *et al.*, 2017).

Atualmente, foi identificada uma região conhecida como ilha ou ponto de resistência AbaR1 com mais de 86.000 pares de bases. Esse fragmento genômico é tido como um dos principais fatores de resistência da espécie (PEREZ *et al.*, 2007). Contudo, nem todas as linhagens de *A. baumannii* apresentam esse padrão genético. A estirpe AYE isolada na França apresenta a ilha de resistência AbaR1, já a linhagem SDF isolada na mesma região não apresenta a mesma característica genética e é suscetível ao tratamento preconizado com β-lactâmicos e tigeciclina (HOWARD *et al.*, 2012). Estudos filogenéticos apontaram ainda que os genes de resistência da ilha de resistência apresentada por *A. baumannii* AYE foram adquiridos por meio da recombinação genética e derivam de linhagens de outras bactérias Gram-negativas, especialmente do gênero *Pseudomonas*, *Salmonella* e *Escherichia*, revelando alta plasticidade genômica (HOWARD *et al.*, 2012).

Além de possuir enzimas inativadoras de antimicrobianos, o genoma de *A. baumannii* apresenta genes responsáveis pela síntese de proteínas de membrana que atuam como bombas de efluxo (ASIF; ALVI; REHMAN, 2018; OPAZO C *et al.*, 2009). Tais mecanismos de efluxo são classificados em famílias, e entre estas é possível citar a bomba de efluxo do tipo *adeABC* pertencente à família RND que é dividida em três partes: (I) uma proteína periférica externa (*adeC*); (II) uma proteína localizada no espaço pericitoplasmático entre as membranas interna e externa atuando como ligação e facilitando a passagem da substância (*adeA*); (III) e uma proteína transportadora periférica interna (*adeB*). Este mecanismo de efluxo atua de forma conjunta sendo sintetizado a partir do cromossomo bacteriano e estando relacionado à maior parte dos casos de resistência apresentado por bacilos Gram-negativos, sendo, portanto, um importante alvo de estudo (ABDI *et al.*, 2020; LI; NIKAIDO, 2004; WIECZOREK *et al.*, 2008). Sabe-se que a atuação dessa bomba de efluxo é ativa e leva ao influxo de prótons (H^+), sendo eficiente para β-lactâmicos, aminoglicosídeos, cloranfenicol, eritromicina, tetraciclina, fluoroquinolonas, tigeciclina e trimetoprima. Sua grande gama de atuação a torna um

importante alvo para redução da resistência (OPAZO C *et al.*, 2009). Contudo, a expressão genética dos genes codificantes das proteínas ABC são dependentes da expressão do gene adeS e adeR (WIECZOREK *et al.*, 2008).

O repertório de resistência de *A. baumannii* tem ainda outras bombas de efluxo relacionadas à resistência, tais como: (I) bomba *abeM* da família *MATE* que se trata de uma única proteína que utiliza da força próton-motora para substituir o uso de energia e levar ao influxo de íons sódio (Na^{++}) e efluxo de fluoroquinolonas; e (II) mecanismos Tet(A), Tet(B) e CmlA que atuam sobre o efluxo de tigeciclina.

A tigeciclina é o único representante da classe das gliciclinas e tem ação na inibição da síntese proteica por se ligar à porção 30S ribossomal. Esse fármaco apresentava boa eficácia contra infecções severas por *A. baumannii*, contudo seu uso ainda não foi padronizado e linhagens resistentes à essa droga emergiram rapidamente (ASIF; ALVI; REHMAN, 2018). Outra alternativa visa como último recurso para tratamento de infecções por *Multidrug Resistant* (MDR) *A. baumannii* é baseada em colistina, uma polimixina que atua desestabilizando a membrana plasmática bacteriana (GARCIA CASALLAS *et al.*, 2019). Contudo, é notória a presença de casos reportados sobre a resistência desse antibiótico bactericida polipeptídio cíclico ao redor do mundo. (DORTET *et al.*, 2018; SNYMAN *et al.*, 2020).

2.3. Fatores de virulência da espécie

Dentre as bactérias de importância nosocomial, *A. baumannii* é uma das espécies de maior relevância (SHRIVASTAVA; SHRIVASTAVA; RAMASAMY, 2017). Fato é que seus fatores de virulência contribuem para sua adaptabilidade e seu sucesso como patógeno (ALIRAMEZANI *et al.*, 2019).

Em comparação com os fatores de resistência, o repertório de fatores de virulência de *A. baumannii* é mais conciso e menos estudado (HARDING; HENNON; FELDMAN, 2018). Contudo, já foram elucidados alguns dos principais mecanismos presentes no genoma da espécie, dentre eles os genes *lasB* responsável codificação da elastase, proteína relacionada à clivagem de elastina do hospedeiro, e o gene *plcN* responsável pela codificação da proteína fosfolipase C, um agente facilitador da invasão celular (ALIRAMEZANI *et al.*, 2019).

Além disso, um fato importante para a patogenicidade microbiana é a capacidade de produção de biofilme. Isso porque sua formação possibilita uma maior sobrevida microbiana em situações de estresse. É possível observar ainda o biofilme como um fator extremamente

relevante para algumas desordens, como endocardite e periodontite, além de possibilitar a sobrevivência microbiana em implantes, cateteres e tubos relacionados à ventilação mecânica (GUPTA *et al.*, 2016). Estudos já relataram a presença de proteína *bap* (proteína de superfície associada ao biofilme) com alta prevalência dentro da espécie *A. baumannii* (BROSSARD; CAMPAGNARI, 2012). Entretanto, mesmo que a presença de *bap* seja importante para o desenvolvimento do emaranhado microbiano, a dinâmica da formação de biofilme é variável de acordo com a linhagem. Relatos anteriores justificam esse fato pela presença de dois genes reguladores: *bfmR* e *bfmS* (TOMARAS *et al.*, 2008).

Apesar de *Acinetobacter* se traduzir de forma literal como "bastão imóvel" e indivíduos do gênero não possuírem flagelo, estudos clássicos já apontaram a presença de diferentes mecanismos de mobilidade dependentes de pili do tipo IV dentro do gênero (CRAIG; FOREST; MAIER, 2019; EIJKELKAMP *et al.*, 2011). Atualmente sabe-se que a motilidade do *A. baumannii* é relacionada à sua adaptabilidade a diferentes sítios anatômicos (VIJAYAKUMAR *et al.*, 2016), e também é considerada um importante fator para sua patogenicidade visto que linhagens mutantes com motilidade reduzida apresentaram também um decréscimo de virulência (PÉREZ-VARELA *et al.*, 2017).

Sendo assim, o conjunto total de genes de resistência e virulência tornam a espécie um patógeno de sucesso. Isso pode ser por vezes justificado pelo fato de ser uma bactéria nosocomial que está associada à capacidade de formar biofilme e resistir à dessecação, sendo esse último fator relacionado à sua origem evolutiva (HARDING; HENNON; FELDMAN, 2018).

2.4. Abordagem patogenômica

Em paralelo ao problema bacteriano houve o advento das tecnologias de sequenciamento de nova geração impulsionando o estudo das ciências ômicas, sobretudo no início dos anos 2000 (HAGEN, 2000). Conceitualmente, estudos genômicos são análises sistemáticas de todo o conteúdo genético de um organismo (WEISSENBACH, 2016). Por se basear em uma metodologia computacional (*in silico*), análises genômicas tendem a ser rápidas e mais baratas em comparação as técnicas clássicas de análise comparativa *in vivo*, sendo escaláveis e independentes de condições de biossegurança laboratorial (SETUBAL; ALMEIDA; WATTAM, 2018). Desse modo, o número de trabalhos genômicos cresceu exponencialmente ao longo dos últimos anos.

Em estudos de microrganismos a genômica se apresenta como uma alternativa para complementar estudos *in vitro* e *in vivo*. Nesse contexto, as análises patogenômicas são em geral análises *in silico* de genomas de microrganismos patogênicos com a finalidade de avaliar e justificar a capacidade de desenvolvimento de determinadas desordens e sobrevivência em condições diversas (SETUBAL; ALMEIDA; WATTAM, 2018; SHEPPARD; GUTTMAN; FITZGERALD, 2018).

Para análises patogenômicas é comum utilizar da informação disponível em bancos de dados públicos com o intuito de comparar e inferir presença de fatores relacionados aos fenótipos de interesse como virulência e resistência. Ainda é possível inferir a história evolutiva de agentes etiológicos microbianos e determinar, por exemplo, diversidade microbiana e proximidade genotípica (SHEPPARD; GUTTMAN; FITZGERALD, 2018; XIAO, Jingfa *et al.*, 2015).

Análises patogenômicas já foram realizadas em diferentes patógenos como: *Pasteurella multocida* (HURTADO *et al.*, 2018); *Pseudmonas aeruginosa* (FRESCHI *et al.*, 2019; JANI; MATHEE; AZAD, 2016), *Corynebacterium pseudotuberculosis* (SOARES *et al.*, 2013) *Klebsiella pneumoniae* (PROFETA *et al.*, 2021); e *Treponema pallidum* (JAISWAL *et al.*, 2020). Por se tratar de uma metodologia de baixo custo e alto rendimento informacional, a patogenômica é uma alternativa para estudos em larga escala de patógenos, como o *A. baumannii*.

3. JUSTIFICATIVA

Acinetobacter baumannii é um dos principais patógenos nosocomiais com distribuição global (PELEG; SEIFERT; PATERSON, 2008; XIAO, DONG *et al.*, 2017). No ano de 2017, a Organização Mundial da Saúde (OMS) publicou uma lista de famílias bacterianas super-resistentes com o intuito de estimular a pesquisa e o desenvolvimento de novas drogas contra essas ameaças. Em uma classificação dada como média, alta e crítica, *A. baumannii* foi classificado como prioridade 1 (crítica) juntamente com *Pseudomonas aeruginosa* e enterobactérias.

De fato, a resistência múltipla de linhagens de *A. baumannii* é um fator crítico para o estudo da espécie (SHRIVASTAVA; SHRIVASTAVA; RAMASAMY, 2017). A constante plasticidade demonstrada ainda pelo genoma da espécie dificulta o fechamento de um repertório de genes de resistência, o que implica em constantes estudos genômicos para determinação de novos elementos agregados tanto ao cromossomo quanto aos plasmídeos desta espécie (HOWARD *et al.*, 2012; IMPERI *et al.*, 2011; PELEG; SEIFERT; PATERSON, 2008). Outro fator relevante é a taxa de mortalidade de pacientes com *A. baumannii*. Artigos apontam que, em Unidades de Tratamento Intensivo, essa taxa varia no intervalo de 26 e 55,7% (FALAGAS; RAFAILIDIS, 2007; XIAO, DONG *et al.*, 2017).

Além disso, infecções por *A. baumannii* podem alcançar diferentes classes sociais e países em desenvolvimento. Sua distribuição global e alta resistência dificultam o tratamento hospitalar em diversos países, sendo relatados surtos epidêmicos em nações como África do Sul, Argentina, Brasil, França, Itália e Estados Unidos. Deste modo, estudos de resistência relacionados à essa bactéria não são limitados por fronteiras geopolíticas sendo necessários e aplicáveis a nível global (PELEG; SEIFERT; PATERSON, 2008).

Ainda nesse contexto, vem crescendo o número de patógenos denominados "superbactérias", e *A. baumannii* é um dos principais organismos modelo no estudo de resistência microbiana (ADEGOKE *et al.*, 2016; ZAVASCKI *et al.*, 2010). Esse fato torna válido o levantamento de seu repertório genômico, sobretudo de genes relacionados à resistência, devido a busca de alvos farmacêuticos para tratamento das infecções.

Por fim, devido aos dados apresentados anteriormente, as comorbidades relacionadas à espécie e taxa de mortalidade relacionada a resistência deste patógeno, o foco deste trabalho foi realizar uma análise aprofundada do repertório de genes de resistência de *A. baumannii*.

4. OBJETIVOS

4.1. Objetivo geral

Realizar um estudo de metanálise e um estudo comparativo acerca do repertório de genes de resistência (resistoma) de 206 linhagens de *Acinetobacter baumannii* através de análises evolutivas e pangenômica da espécie.

4.2. Objetivos específicos

- Investigar a resistência à carbapenem de linhagens de *Acinetobacter baumannii* a partir de análises estatísticas de estudos publicados metanálise);
- Analisar o posicionamento filogenético da espécie dentro do gênero *Acinetobacter*;
- Montar e analisar a reconstrução filogenômica das 206 linhagens de *A. baumannii*;
- Classificar as linhagens de acordo com a similaridade genômica;
- Realizar a análise pangenômica da espécie, bem como a plasticidade genômica das linhagens;

5. RESULTADOS E DISCUSSÃO

5.1. Estudo de metanálise de *Acinetobacter baumannii*

Os resultados da metanálise realizada encontram-se no artigo intitulado "*Acinetobacter baumannii and its relationship to carbapenem resistance: a meta-analysis*" que foi submetido em junho de 2021 na revista Multidisciplinary Digital Publishing Institute (MDPI). Nesse trabalho foi abordada a resistência de linhagens de *A. baumannii* à carbapenem em diferentes estudos com o intuito de caracterizar a significância de resistência dessa espécie quando comparada com outros táxons. Foi abordado inicialmente um número de mais de 90 mil trabalhos que após uma seleção sistemática foi reduzido à oito trabalhos elegíveis.

Optamos por inserir os resultados da metanálise antes dos resultados apresentados no artigo "*Pan-resistome insights into the multidrug resistance of Acinetobacter baumannii*" para elucidar melhor o aspecto introdutório à problemática estudada e relatada na Introdução e Justificativa deste trabalho.

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Review

Acinetobacter baumannii and its relationship to carbapenem resistance: a meta-analysis

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Abstract: Infections by antibiotic-resistant bacteria are a major and complex global health issue. In this context, *Acinetobacter baumannii* is particularly important because of its ability to withstand treatments by β-lactams such as carbapenem. The objective of this work was to investigate, through systematic analysis and meta-analysis, the chance of resistance to carbapenem in *A. baumannii* strains. For this, a search was conducted for the PubMed and Cochrane databases based on the key words: "*Acinetobacter baumannii*" AND "beta-lactam" OR "penicillin" OR "cephalosporin" OR "cephamycin" OR "carbapenem" OR "monobactam". The initial search resulted in a total of 90,475 articles. It was filtered based on eligibility criteria and eight articles were selected for analysis. An Odds Ratio value equivalent to 3.55 was obtained, indicating a high chance of resistance to the carbapenem of strains of the species. Therefore, it is supposed that *A. baumannii* infection cases have a high chance of not responding adequately to treatments based on carbapenem.

Keywords: *Acinetobacter baumannii*; carbapenem-resistance; CRAB; meta-analysis.

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1. Introduction

Acinetobacter baumannii is a Gram-negative coccobacillus, catalase positive, oxidase negative [1]. According to the World Health Organization, it is one of the most important nosocomial pathogens, in addition to being a great model of resistance to antimicrobials [2]. This organism is related to several comorbidities such as pneumonia [3], bacteremia [4], cystitis [5], meningitis [6], among others. It also has increasing relevance in one health [7].

With the turn of the century, *A. baumannii* acquired fame for its exacerbated resistance to several classes of antimicrobials, among them β-lactams. The origin of its resistance is still mysterious, but it is known that its mechanisms derive in part from elements derived from the horizontal gene transfer (HGT) from species belonging to the genera *Pseudomonas*, *Klebsiella* and *Salmonella* [8]. Another justification for its resistance is the presence of elements related to the evolutionary origin of the species that remained in the genome and showed an adaptive advantage in a hospital environment [9–11]. Especially within the β-lactam class, the species has a higher degree of resistance to carbapenem. This fact culminated in creating the term CRAB (carbapenem-resistant *Acinetobacter baumannii*), which is widespread in academia and life sciences [2].

Recent studies indicate that the best treatment options in the case of CRAB infections are based on colistin and tigecycline, or pharmacological associations with sulbactam [12,13]. However, there are case reports where these strategies have not been effective [14,15]. Nevertheless, due to the importance of the pathogen, there must be a constant review and survey of scientific data in order to design the development of pathogenic factors and outline the best treatment strategies.

In this context, the meta-analysis is a statistical strategy that aims to combine the results of different studies in single effect size with greater significance than individual studies [16]. In other words, it is a tool capable of treating several studies as single research and considers the result extracted from each population to produce a more significant effect size [17]. Thus, it composes an excellent statistical model to deal with data obtained from isolated studies and corroborate with making biological conclusions. For this reason, it is an adequate research strategy for the analysis of multiple case studies that deal with events of resistance and susceptibility of strains of the species.

Therefore, this study aimed to investigate the chance of resistance to carbapenem of the species *A. baumannii*, based on data prospected from clinical studies and summarized by a meta-analysis strategy.

2. Materials and Methods

Search Strategy

A bibliographic search was carried out based on the databases Cochrane Central Register of Controlled Trials, which contains one of the largest repositories of clinical studies globally, and PubMed, which is one of the largest databases of articles and publications in the world. The search was limited to articles published from 2000 to August 16, 2020, with no language restrictions for studies carried out in adult humans. All available comorbidities were considered. To frame articles related to the research point, the following keywords were used: "*Acinetobacter baumannii*" AND "beta-lactam" OR "penicillin" OR "cephalosporin" OR "cephamycin" OR "carbapenem" OR "monobactam".

Selection criteria

The types of studies included in the analysis were clinical and laboratory reports in which there were reports of the presence of *A. baumannii* strains resistant to β-lactam classes. An in-house script was developed in Python 3 to perform specific filtering of abstracts. Through this, studies that do not mention the interest species in the abstract, title or keywords were excluded. Studies that deal exclusively with *A. baumannii* strains have been excluded, requiring data from other bacteria concurrently. This criterion was applied because the odds ratio statistics is based on the proportion and requires at least two distinct groups with subgroups. Bacteria were considered resistant if the report presented: (I) microbial susceptibility test indicating resistance; (II) isolates named as antimicrobial-resistant (i.g.: carbapenem-resistant *Acinetobacter baumannii* CRAB, and carbapenem-resistant *Pseudomonas aeruginosa* CRPA); (III) failure of clinical outcome defined by non-microbiological eradication of the pathogen or failure to resolve clinical signs and symptoms.

Data extraction

The quantities required to carry out the study were previously selected through discussion between all authors. A standardized table was built to organize the selected data. The first author extracted the following data from each study based on the standardized matrix: study title, number of resistant *A. baumannii* isolates, number of susceptible *A. baumannii* isolates, number of resistant isolates from bacteria other than *A. baumannii*, number of susceptible isolates from bacteria other than *A. baumannii*, dosage applied, class of antimicrobial used, author, year of study development, publication journal, country where the study was developed.

Data analysis

Statistical analysis was developed entirely in R Studio. The meta-analysis was performed comparing the reasons for the chances of resistance to β-lactams if the pathogen is *A. baumannii* and if it is not *A. baumannii*. The heterogeneity of the effects of the studies was assessed using I^2 statistics. I^2 value> 75% accompanied by a significance value <0.05 was used to consider high heterogeneity. In addition, the outlier test was

performed using the GOSH function based on the k-means, DBSCAN and Gaussian Mixture Models algorithms. To summarize the effects, the Random-Effects-Model was selected because it is considered the most suitable for analysis in life sciences. Publication bias was assessed using a funnel plot, and if ten or more studies were added to the research, the Egger's test would be used. For the correlation analysis, Pearson's statistics were used.

3. Results

3.1. Studies selection and characteristics

Prospecting started with 6,019 articles derived from the Cochrane platform and 84,726 studies derived from the PubMed platform. After the first successive filtering, 2,516 articles remaining on the Cochrane platform and 509 articles on the PubMed platform remained in the analysis. After removing redundancy, 2,576 articles remained in the analysis. Subsequently, as a result of the specific abstract filtering, 86 papers were selected for analysis. Finally, eight articles were considered eligible for meeting the inclusion criteria [18–25] (Figure 1).

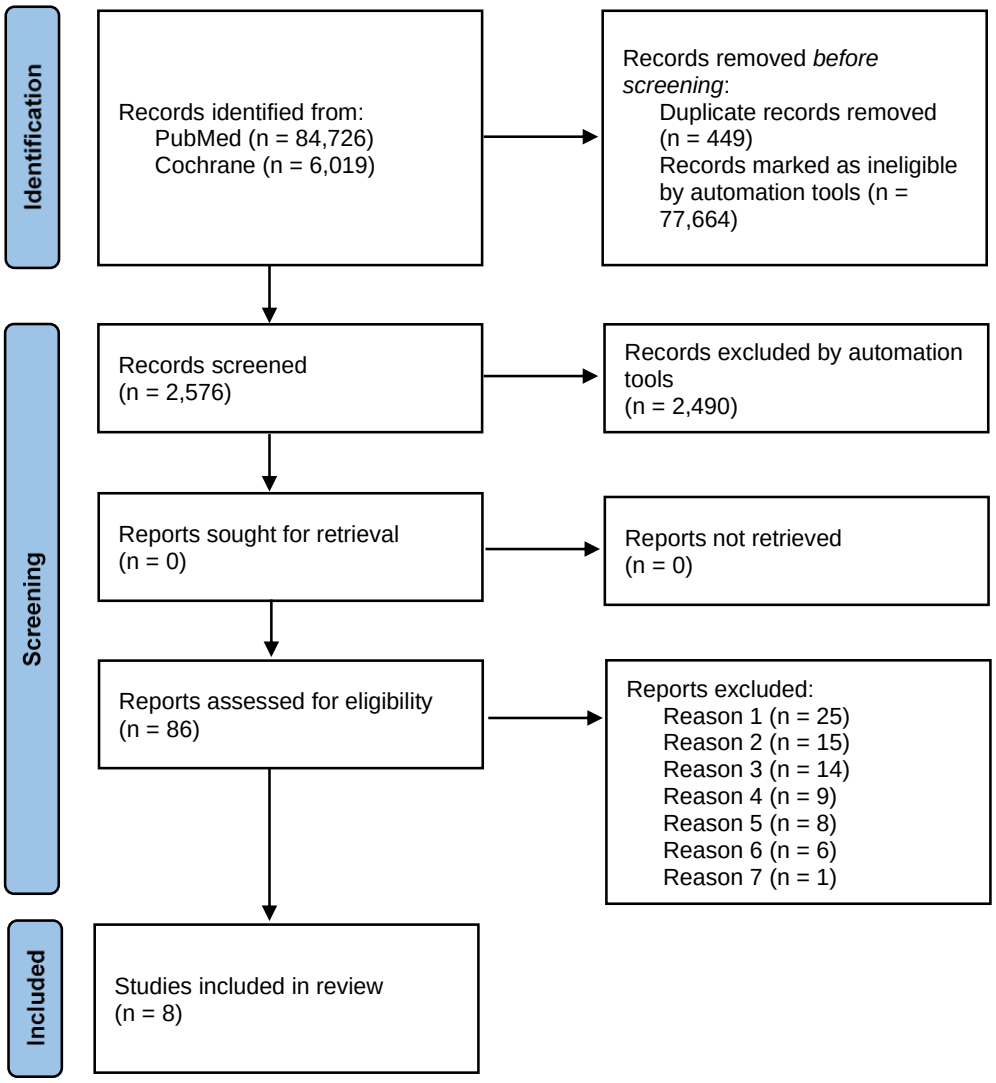


Figure 1. Systematic representation of selecting the studies according to the previously established filtering criteria.

Among the reports excluded from the analysis: 25 did not present clear data regarding the prevalence of resistant *A. baumannii* strains; 15 did not harbored β-lactams; 14 do not present published results; 9 do not address *A. baumannii* as at least one of the species raised; 8 items are unavailable; 6 do not show conclusive results (see Figure 1). Only 1 study addressed any β-lactam other than carbapenem, so the analysis was performed based on evidence of resistance only to carbapenem.

Data were extracted from the selected studies regarding the number of resistant and susceptible isolates in each case. The data distribution is shown in Table 1.

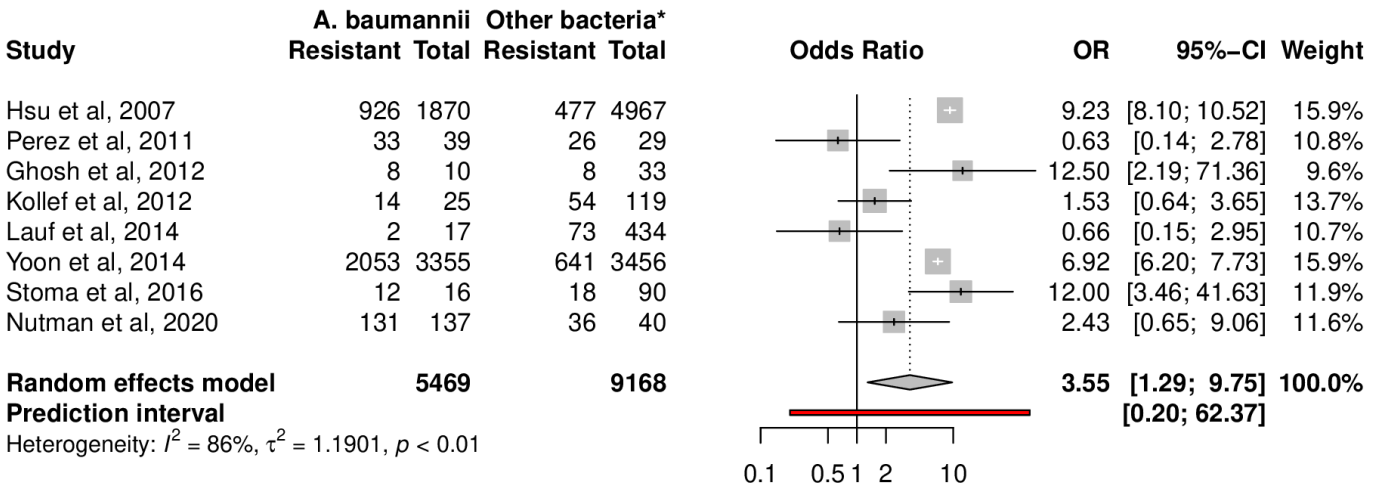
Table 1. Distribution of data extracted from each study is considered eligible for the development of the analysis and their respective values.

Author	Resistant n (%)		Susceptibility n (%)		Year	Country
	Acinetobacter baumannii	Other bacteria*	*Acinetobacter baumannii*	Other bacteria*		
Hsu et al, 2007	926 (49.5)	477 (9.6)	944 (50.5)	4490 (90.4)	2007	Singapore
Perez et al, 2011	33 (84.6)	26 (89.6)	6 (15.4)	3 (10.4)	2010	USA
Kollef et al, 2012	14 (56)	54 (45.4)	11 (44)	65 (54.6)	2012	Whole world
Ghosh et al, 2012	8 (80)	8 (24.2)	2 (20)	25 (75.8)	2012	India
Yoon et al, 2014	2053 (61.2)	641 (18.5)	1302 (38.8)	2815 (81.5)	2014	South Korea
Lauf et al, 2014	2 (11.8)	73 (16.8)	15 (88.2)	361 (83.2)	2014	Europe, USA, Canada, Latin America, Asia, India, Australia, and South Africa
Stoma et al, 2016	12 (75)	18 (20)	4 (25)	72 (80)	2016	Belarus
Nutman et al, 2020	131 (95.6)	36 (90)	6 (4.4)	4 (10)	2020	Italy, Greece, and Israel

***Other bacteria:** *Enterobacter cloacae*, *Enterococcus faecalis*, *Escherichia coli*, *Klebisiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*, *Streptococcus agalactiae*.

3.2. Statistical analysis and synthesis

As a result of the statistical analysis, comparisons were made between the amounts of resistant and susceptible to carbapenem isolates in both raised groups, being categorized as group Ab (*Acinetobacter baumannii*) and group Ob (Other bacteria). Comparisons were made due to the odds ratio of resistance if the isolate is *A. baumannii*. Figure 2 represents the individual results of each study, the values considered for the analysis of heterogeneity and the final result of the summary of the results.



• **Figure 2.** Forest plot that summarizes the results of the analysis by odds ratio of the selected studies. *Other bacteria: *Enterobacter cloacae, Enterococcus faecalis, Escherichia coli, Klebisiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Stenotrophomonas maltophilia, Streptococcus agalactiae*.

It is observed that four studies have a value of 1 within the confidence interval found considering p-value <0.05, which are the works of Perez et al. (2011), Kollef et al. (2012), Lauf et al. (2014) and Nutman et al. (2020). In contrast, two studies presented the most significant weight for the final prediction, being the work of Hsu et al. (2007) and Yoon et al. (2014). This fact is related to the size of the samples taken by the authors.

As a result of the analysis by the random-effects model, the carbapenem resistance index of *A. baumannii* strains was significantly higher than in other bacterial species (OR 3.55, 95% CI 1.29–9.75). This result points out that *A. baumannii* strains have about 355% chance of resistance to carbapenem when compared to the of cases of resistance of other species. These data suggest that carbapenem-based monotherapies have a lower success rate in *A. baumannii* strains.

In the heterogeneity analysis, an I^2 value equivalent to 86% was obtained with a p-value < 0.01. This fact implies a high heterogeneity between studies. In contrast, τ^2 is equal to 1.1901, which corroborates the previous heterogeneity statement. However, it is worth noting that both I^2 and τ^2 statistics gain greater statistical power when ten or more studies are added to the analysis. However, even considering the high heterogeneity, the random removal of studies from the final analysis did not show a significant reduction in the heterogeneity values (see Supplementary Figure S1).

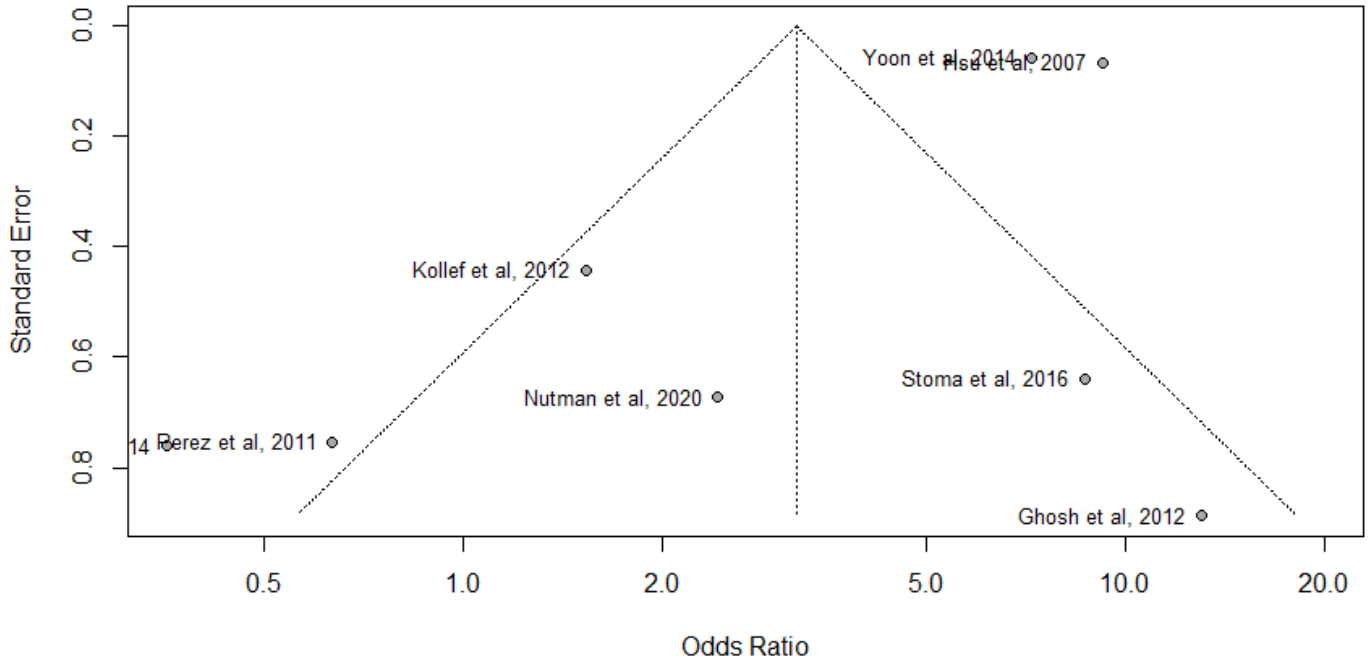
As a result of the correlation analysis, an r value equivalent to 0.748 was obtained (p-value = 0.043) (see Supplementary Figure S2). This indicating that there is a correlation between the advance of years and the increase in the percentage of carbapenem-resistant *A. baumannii* isolates. For this result, the alternative hypothesis considered was that the correlation is greater than 0. This fact indicates that the prevalence of resistant isolates of the species increased over the years.

3.3. Publication bias analysis

In the analysis of publication bias, the Egger's test was performed, resulting in a p-value = 0.127, which would suggest the absence of asymmetry in the funnel plot. However, the number of studies added is less than 10 (k = 8). This fact significantly reduces the significance of the Egger's test result, making its statistical support just illustrative.

Figure 3 represents the funnel plot built to analyze the symmetry of the data distribution. Through Duval and Tweedie's trim-and-fill procedure, it was possible to observe that by adding only two studies with high standard error and high effect size, it

would be possible to recover the symmetry of the graph if the p-value of the Egger's test was inferior to 0.05.



- **Figure 3.** Funnel plot represents the distribution of data extracted individually from each study and their spatial positions around the calculated standard error.

4. Discussion

An essential finding of the study is the value of summarizing the studies. This result shows that *A. baumannii* has more than times the chance of resistance to carbapenem from other bacteria related to hospital infections.

The carbapenem resistance of *A. baumannii* comes mainly from carbapenemases and efflux pumps. Class D β-lactamases (oxacillinases) are part of the major epidemiological problem of the species. This is because its expression implies a significant increase in resistance to carbapenem and cephalosporins. [26,27]. Among the enzymes, the most discussed and represented are OXA-23, OXA-24 and OXA-58 [28,29]. Taking this fact into account the OXA-23 enzyme is prevalent in 60.76% of the complete sequenced *A. baumannii* genomes available at NCBI [30], however, studies have already pointed out a prevalence above 90% in certain regions [31]. In contrast, OXA-24 and OXA-58 have a prevalence of respectively 3.13% and 1.47% of the complete sequenced genomes of *A. baumannii* available at NCBI [30]. However, studies have also pointed out the presence of OXA-23 and OXA-24 in *P. aeruginosa*, another great resistance model, with a prevalence of 11.19% and 2.24%, respectively [32].

Among efflux pumps, it is known that their action against multiple classes of antimicrobials is one of the main factors of resistance to broad-spectrum drugs. In the case of *A. baumannii*, the presence and expression of efflux systems such as AcrAB-TolC significantly reduce susceptibility to antimicrobials, including carbapenems [33,34].

In general, in previous studies, a prevalence of approximately 80% of *A. baumannii* strains resistant to meropenem was observed, while *Pseudomonas aeruginosa*, another significant resistance model, showed about 20% of strains resistant to the same drug [35]. Still dealing with strains of the genus Acinetobacter resistant to carbapenem, there have been reports of prevalence above 90% [36]. All these data corroborate with the result raised of high probability resistance of the species.

This study has some limitations. The number of eligible studies is low, which reduces the significance of the analysis of publication bias. Information from β-lactams other than carbapenem was not included because insufficient data on resistance and susceptibility related to *A. baumannii* and other species were found simultaneously. The correct identification of the pathogen species is a major bias agent considering that it is not possible to guarantee that the identification performed by the researchers is accurate.

5. Conclusions

In conclusion, this study presents results corroborating the clinical and epidemiological data already presented by major global health agencies. In a general field, it was observed that infection cases associated with *A. baumannii* are more likely to be inefficient for carbapenem treatments. Based on this fact, the prerogative of detailed analysis of microbial sensitivity tests is reiterated as an essential tool to deal with resistance conditions in the clinical scope and the improvement of antimicrobials consumption practices and its administration. Further studies are needed to verify and corroborate our findings, especially considering other classes of antimicrobials. It would be interesting to observe systematic analysis results of the new classes of preference for treatments against *A. baumannii*, such as tigecycline and colistin.

Supplementary Materials: The following are available online at https://drive.google.com/drive/folders/1xZ__jIZR0eEOCQhucs21XxsL822EUIYR?usp=sharing, Figure S1: Graph representing the I^2 values obtained by the analysis after consecutive removal of random studies. Note that in no case was the value of I^2 lower than 0.80. Figure S2: Distribution graph of the percentage of resistant *A. baumannii* isolates over the years. The trend curve is based on Pearson correlation.

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5.2. *Acinetobacter baumannii*: pan-resistoma e evolução

No trabalho em questão é abordado o repertório genético total da espécie, com enfoque em mecanismos de resistência aos antimicrobianos, e uma breve passagem por vias de adaptação do patógeno. Também é apresentada a plasticidade genômica das linhagens em estudo, bem como as disparidades genômicas relacionadas à tipagem molecular, como também filogenômicas encontradas e relacionadas à epidemiologia do organismo modelo. Os principais resultados encontram-se no artigo publicado na revista *Antibiotics* ISSN 2079-6382 em maio de 2021, sendo intitulado "*Pan-resistome insights into the multidrug resistance of Acinetobacter baumannii*".

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Article

Pan-Resistome Insights into the Multidrug Resistance of *Acinetobacter baumannii*

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Abstract: *Acinetobacter baumannii* is an important Gram-negative opportunistic pathogen that is responsible for many nosocomial infections. This etiologic agent has acquired, over the years, multiple mechanisms of resistance to a wide range of antimicrobials and the ability to survive in different environments. In this context, our study aims to elucidate the resistome from the *A. baumannii* strains based on phylogenetic, phylogenomic, and comparative genomics analyses. In silico analysis of the complete genomes of *A. baumannii* strains was carried out to identify genes involved in the resistance mechanisms and the phylogenetic relationships and grouping of the strains based on the sequence type. The presence of genomic islands containing most of the resistance gene repertoire indicated high genomic plasticity, which probably enabled the acquisition of resistance genes and the formation of a robust resistome. *A. baumannii* displayed an open pan-genome and revealed a still constant genetic permutation among their strains. Furthermore, the resistance genes suggest a specific profile within the species throughout its evolutionary history. Moreover, the current study performed screening and characterization of the main genes present in the resistome, which can be used in applied research to develop new therapeutic methods to control this important bacterial pathogen.

Keywords: antimicrobial; drug resistance; pan-genome; multilocus sequence typing; nosocomial infections

1. Introduction

Acinetobacter baumannii is a Gram-negative bacterium, aerobic, non-fermenting, catalase-positive coccobacillus with cosmopolitan distribution [1,2]. Most of the clinical cases involving this bacterial species are related to one or more of the following pathological conditions: severe pneumonia, meningitis, bacteremia, and erysipelas [1,3–5]. Although members of the genus *Acinetobacter* are ubiquitous, they are rarely isolated in the environment outside of hospitals, even during outbreaks [6].

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This species has several intrinsic resistance mechanisms, such as (I) the presence of β-lactamases, which is responsible for the degradation of β-lactam drugs; (II) the presence of multiple drug efflux pumps that prevent the increase in the concentration of antimicrobials in the cytoplasm; (III) changes in the molecular pattern of proteins associated with plasma membrane; (IV) ribosomal methylation, which hinders the action of antimicrobials related to the regulation of protein translation processes, such as tigecyclines and quinolones; and (V) the presence of enzymes capable of degrading multiple antimicrobials [7–9].

The recommended treatment usually prescribed for infections with *A. baumannii* is based on β-lactam antibiotics, such as cephalosporins and carbapenems [10]. This class of antibiotics interferes with peptidoglycan biosynthesis and avoids forming the cell wall [11,12]. Nonetheless, over time, because of its high adaptation skills, strains capable of resisting the high concentrations of these antimicrobials have been detected [2,8]. Such cases of resistance can be classified into three categories: (i) Extensively Drug Resistant (XDR) refers to when it is resistant to more than three classes of antimicrobials; (ii) Multidrug Resistant (MDR), when it is resistant to almost all the antimicrobials except for two; or (iii) Pandrug Resistant (PDR), when it is resistant to all known antimicrobials.

Because of all these factors associated with the ability of *A. baumannii* to survive to adverse conditions (grow under a wide thermal range and in an environment with low concentrations of nutrients) and the resistance exhibited by *A. baumannii* generates numerous obstacles for the hospital treatment team, making it difficult to treat patients [1,4,6,9,13]. Some resistance mechanisms of protein origin have been previously evidenced, such as changes in the DNA-gyrase complex and an increase in the expression of the *ampC* that confers carbapenem resistance to *A. baumannii* [8].

In order to better clarify some of the resistance mechanisms present in the species genome, this study explores the genes occurring on the resistome of 206 complete genomes of strains of *A. baumannii* that are related to the resistance of this species by using multi-omic methodologies for comparative genomics, phylogenomics, and the pan-resistome of this species.

2. Results

2.1. Genomic Analysis and Geographic Distribution

Acinetobacter baumannii is a genetically diverse bacterial species and there is a variety of typing methods to identify genetic differences among the strains that could be associated with pathogenicity, epidemiological origin, dissemination, and evolutionary patterns [14]. Sequence type and phylogenetic analysis allow for the identification of genotype groups with a phylogenetic relationship and explore the diversity among the strains [15]. Similarity nucleotides and MLST analysis with geographical data can reveal a better knowledge of the epidemiological context and population structure among the strains around the world [16,17]. With the analysis of genomic similarity based on sequence alignment and geographic distribution, it is possible to infer bacterial clonality, considering that strains of bacterial species isolated from the same region tend to have the same genic repertoire. Even though events of gene drift and vertical gene transfer cannot be ruled out, genetic characteristics are generally conserved when dealing with isolated bacteria in the same site or nearby sites.

Numerous epidemiological studies of *A. baumannii* associate it with the presence of ST by local origin, as seen in the occurrence of ST 848 (CC 208) (Oxford scheme) carrying resistance gene to carbapenems in India [18], and likewise the frequent presence of ST15, ST25, ST79, and ST1 in South America [19,20]. A recent phylogeographical analysis of the Italian isolates belongs to the only clonal group ST78 (Pasteur scheme) [14].

The 206 *A. baumannii* complete NCBI genomes sequences were analyzed (see Supplementary Table S1). The genomes have sizes varying from 3.48 Mb to 4.43 Mb, with a genomic GC content of 39.05%. Considering that nearby isolated bacterial genomes tend to maintain the same genetic characteristics, the study of the geographical distribution of

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A. baumannii is an essential method for evaluating the conservation of the species in the global context.

It is important to note that all strains added to the study showed similarities greater than 95% based on the ANI results (see Supplementary Figure S1). This result corroborates the statement that all strains belong to the same species [21,22]. A total of five relevant clusters with high similarity (≳98.5%) belonging mainly to specific STs (1, 2, 10, 79, and 437) were retrieved. This finding corroborates the conservation of genomes belonging to the same ST. Consequently, strains related to the same ST were expected to be isolated at locations to justify the high genomic similarity. Nevertheless, the geographic distribution of the strains according to the ST proved to be misplaced. Considering that different STs were isolated on distinct continents, possible factors that could justify this misplacing are microbial ubiquity and globalization (Figure 1). A higher number of deposited genomes belong to ST 2 (50% of the used dataset) and a more significant number of strains were isolated from the Asian continent (51.2% of the used dataset). These data do not corroborate the epidemiological information on the distribution of outbreaks caused by the bacterium *A. baumannii* [9,15,18]. Thus, this concludes that there is a more significant number of sequencing performed on the Asian and North American continents since epidemiological outbreaks have been reported in several developing countries over time (Argentina, Brazil, and South Africa). Furthermore, this pathogen has also reported outbreaks of infections on the European continent; however, the number of isolates from that continent is still much lower.

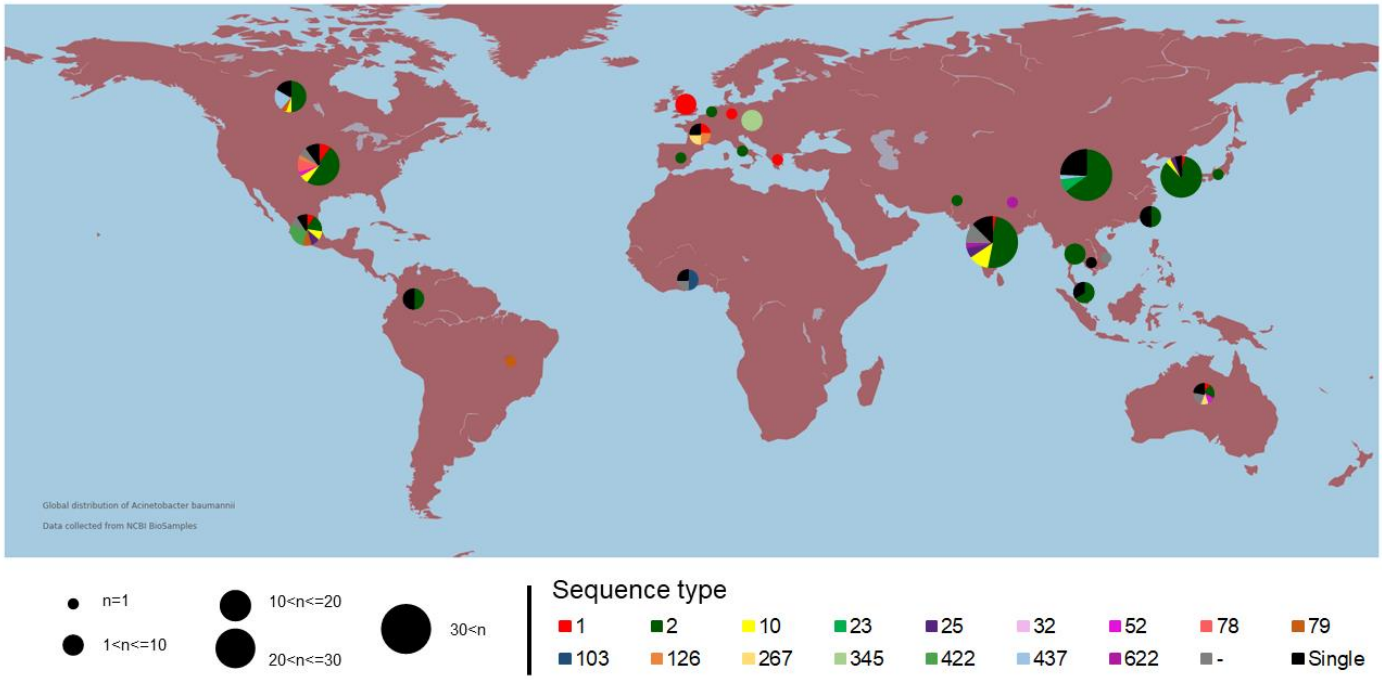

Figure 1. Graphical representation of the global distribution of isolation sites of different strains of *Acinetobacter baumannii* in a grouped way. The colors represent the sequence types of the strains in this study. The size of the circle indicates the number of isolated strains.

2.2. Phylogeny and Phylogenomics

Phylogenetically, all the *A. baumannii* strains were grouped in the same clade within the *Acinetobacter* genus, confirming the monophyly of this species (see Supplementary Figure S2). This result also points out that the *A. baumannii* strains are highly conserved within the species. It is also observed in different microbial species and is consistent with reports from the literature on phylogenetic analysis, indicating that the use of housekeeping genes to infer evolutionary history is a good qualifier of phylogenetic distance and epidemiology [23].

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Three strains (FDAARGOS_494, FDAARGOS_493, and FDAARGOS_560), previously identified as *Acinetobacter* sp., were grouped together and inside the *A. baumannii* clade, strongly suggesting that they are, in fact, of this same species. This taxonomic re-classification has already occurred in other cases of bacterial species [24–26]. More phylogenomic studies, including tetranucleotide analyses, Average Nucleotide Identity (ANI), and the presence and absence of species-specific genes evaluation, are needed to confirm this hypothesis and assure taxonomic reclassification based on genomic data and theoretical background [24,27]. These three strains were not added to the subsequent analyses. The genomic similarity analysis integrated with a previous phylogenetic analysis was ideal for determining the exclusive addition of *A. baumannii* strains to the following in silico analysis, ensuring that the pan-genomic analyses were not skewed.

The *A. baumannii* strains were grouped according to their respective STs in the phylogenomic tree, using the core genome sequence (Figure 2). Nonetheless, in the phylogenomic analyses, the ST 2 strains (represented in green) formed paraphyletic clades, and, thus, these strains cannot be considered to be in the same group. The strains represented in gray do not have a defined ST, but they all grouped in the same clade, indicating the high similarity among them (see Supplementary Figure S1).

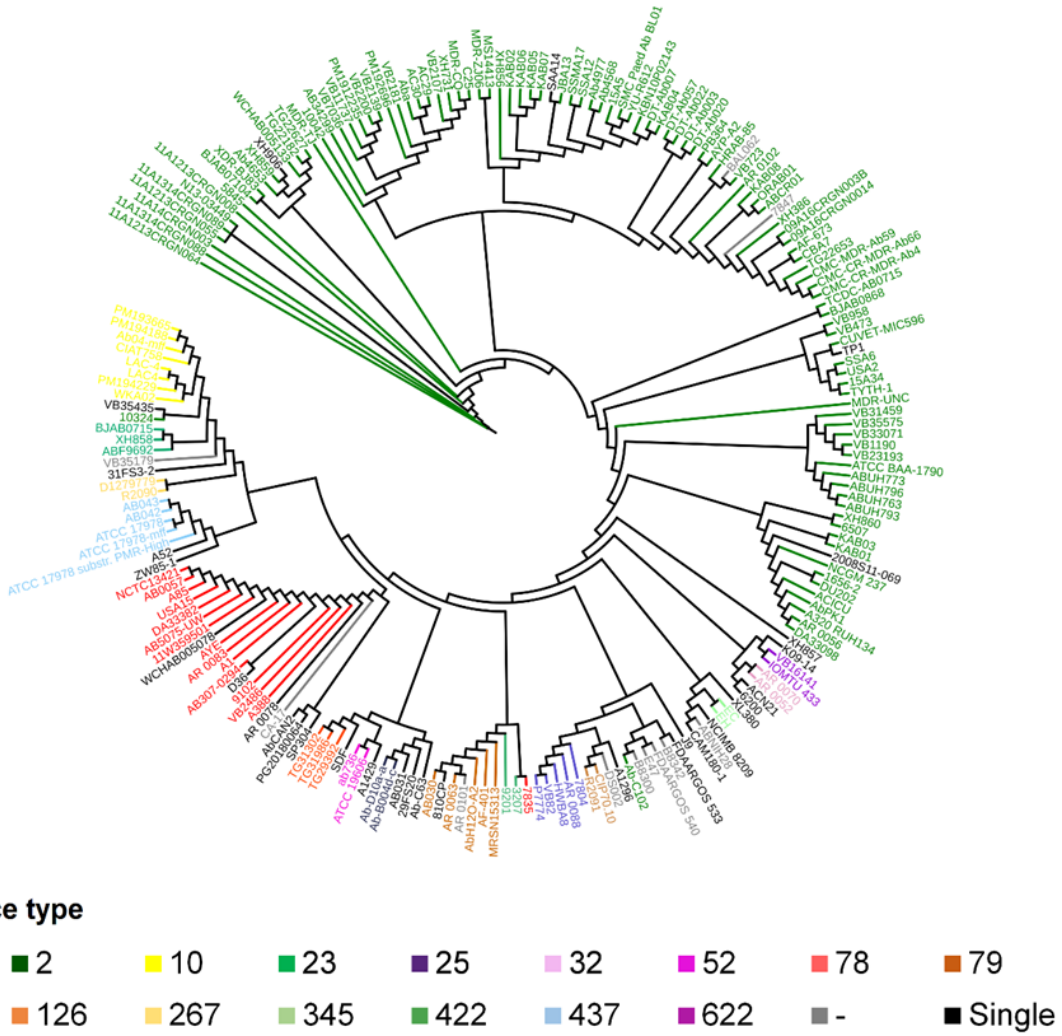

Figure 2. Phylogenomic tree based on the core genome of 206 *Acinetobacter baumannii* strains. The colors represent the grouping by sequence type. The method used was maximum likelihood with statistical support of 1000 bootstraps with 1999 genes present in the core genome.

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2.3. Genomic Plasticity

During the analysis of genomic plasticity, a significant gap in the *A. baumannii* strains could be observed when visually compared. Even strains belonging to the same ST were not identical, although they were genomic, phylogenomically closer, and shared the same clade. This result suggests that the strains of this species are not very clonal and tend to have a high rate of gene permutation since there are many gaps between the genomes (Figure 3).

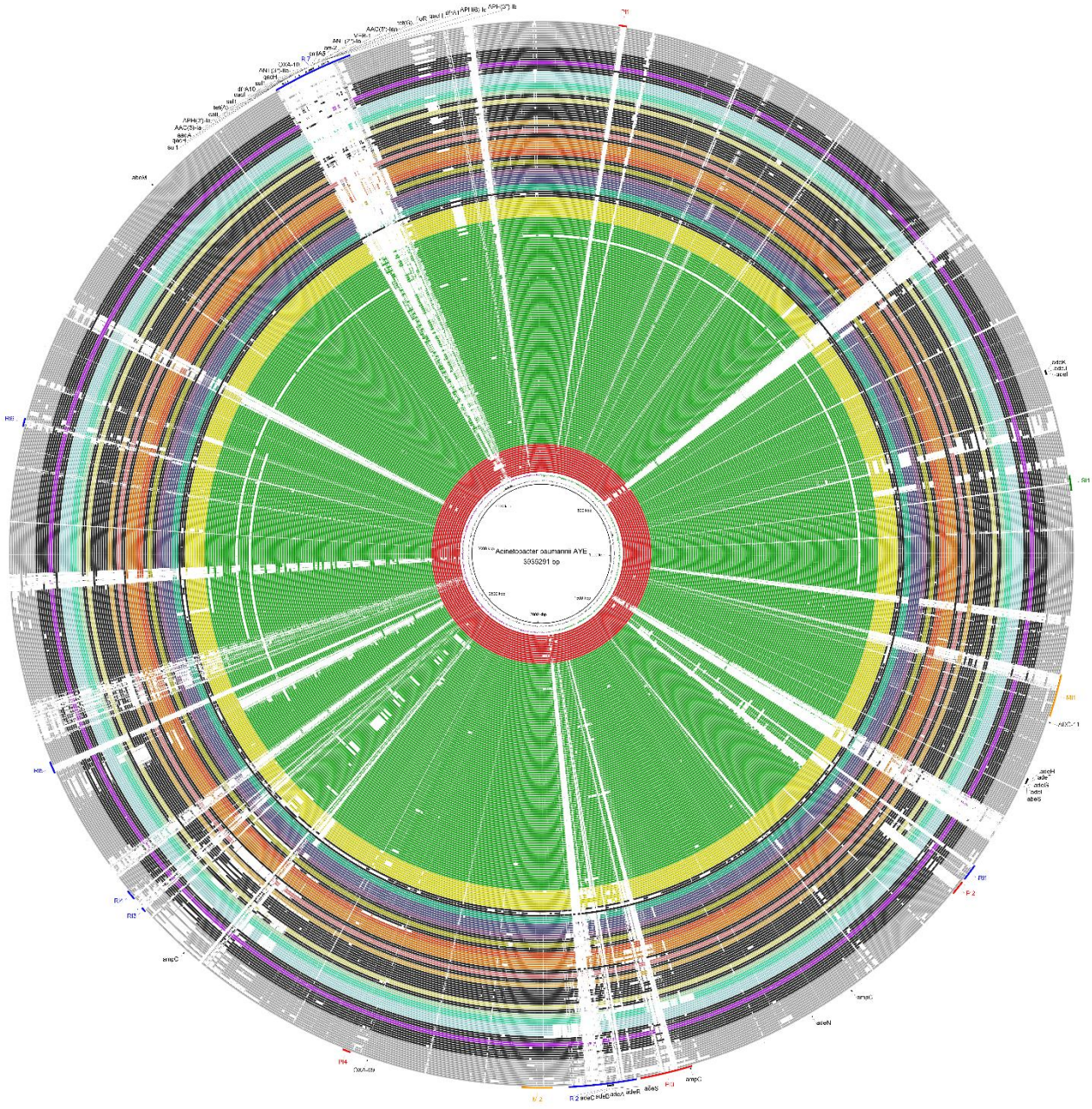

Figure 3. Representation of the circular genome of the *A. baumannii* AYE strain as a central genome. The compared strains were grouped and colored.

Comparative genomic analyses of the 206 *A. baumannii* genomes, using the AYE strain as a reference, showed the presence of 14 genomic islands (Figure 3). Among these 14 genomic islands, 4 were Pathogenicity islands, 2 were Metabolic islands, 1 was a Symbiotic island, and 7 were Resistance islands. Furthermore, 1 full-sized Resistance

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island (RI7 or AbaR1) was identified within the AYE strain. This genomic region has a length of 96,878 nucleotides and contains the highest amount of resistance genes found in this species. There are 25 resistance genes within this island divided into efflux pumps and proteins with enzymatic activity.

The islands RI2 (80,220 bp) and RI7 (96,878 bp) were conserved within the species, which were more present within strains belonging to ST 1. Outside of this cluster, however, both islands were not entirely found. A similar result was observed in smaller islands, such as RI1 (20,317 bp), RI3 (6077 bp), RI4 (12,534 bp), RI5 (14,763 bp), and RI6 (10,374 bp), indicating that they are unstable regions within the genome.

There is a great number of genomic islands for the *A. baumannii* species, which reveals its high genomic plasticity. Although we identified a reduced number of type sequences and phylogenetically close strains, analyzing the complete genomes showed how all the strains are different in their gene content. This could be due to the horizontal acquisition of mobile genetic elements or gene duplication events.

2.4. Analysis of the Pan-Genome for Understanding this Species

There is an intensive effort to know the total repertoire of the *A. baumannii* species. Classically, the pan-genome assesses the total gene repertoire of a sample, population, or species. To this end, it considers subpartitions of the complete set, which are (I) a core genome consisting of genes shared by all the strains analyzed; (II) an accessory genome consisting of genes shared by two or more strains analyzed, but not all strains; (III) singletons (or exclusive genes), characterized to present exclusively in a single strain [28].

As a result, according to the Heaps' law, the pan-genome of *A. baumannii* remains open (α = 0.71), which by each newly added genome, the number of new genes will increase the genetic repertoire of the species. This result was obtained using the formula $n = \mathrm{a} \times x^{1-\alpha}$, where n is the estimated size of the pan-genome for a given number of genomes, x is the number of genomes used, and α is a fitting parameter [28]. As a rule, when $0 < \alpha < 1$, the pan-genome is considered open. This fact also corroborates the high genomic plasticity already reported for this species, especially considering that this bacterium has an exceptional ability to obtain new gene content through transposable elements [14,29].

The pan-genome analysis revealed a total of 12,336 genes, of which 1999 genes are shared for all strains (complete genome sequences of *A. baumannii*), and 3920 were strain-specific genes. The accessory genome, except for single genes, is made up of 6417 genes. Figure 4 represents the development of the *A. baumannii* pan-genome. It is possible to observe that even using 206 genomes, the curve did not reach a point of stability or a plateau. This fact corroborates the alpha value found, as it also indicates an open pan-genome.

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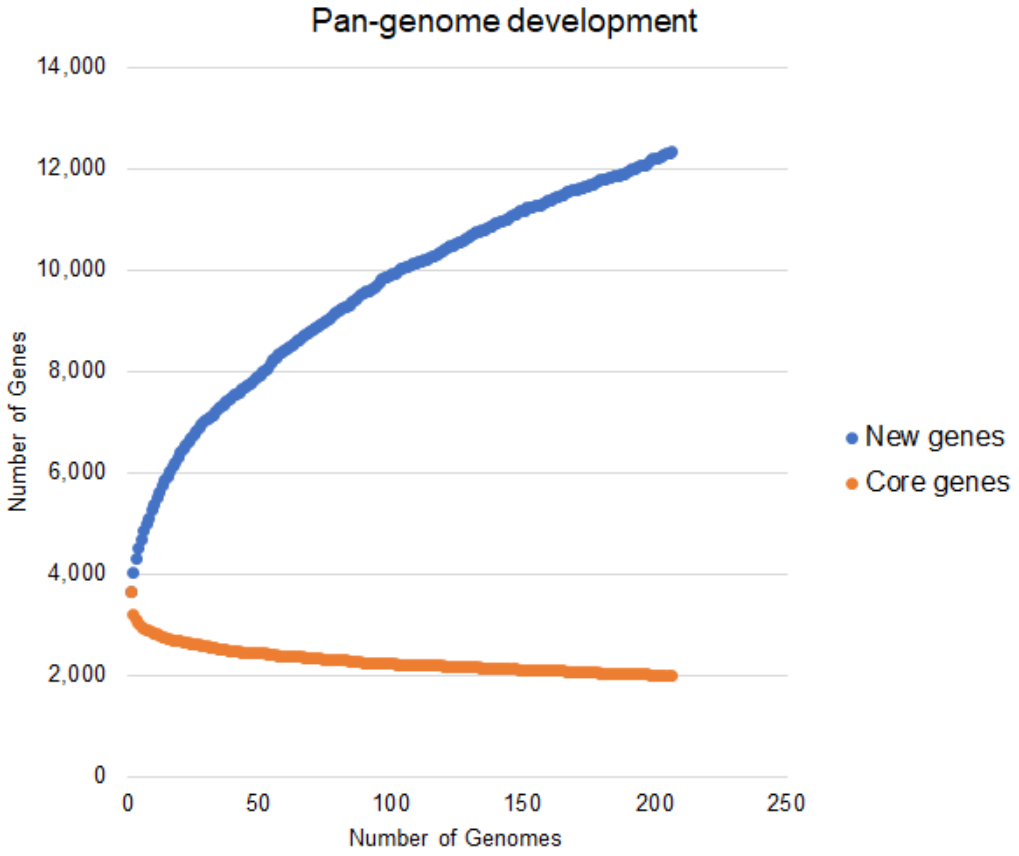

Figure 4. Development curve of the pan-genome of *Acinetobacter baumannii*.

The different patterns of the presence of genes of the SDF strain can be observed in a detailed analysis. This strain is already known to be susceptible to antimicrobials and is the only representative of sequence type 17. Its accessory gene pattern differs from all the others and has about 362 unique genes, which contrasts with the pattern of the super-resistant AYE strain, which contains about 11 unique genes. This fact, combined with the distant phylogenomic position of the strain, shows how different the susceptible strain is from the others.

A more accurate analysis of the total pan-genome indicates the number of genes related to specific bacterial metabolic pathways. Such analysis is based on the KEGG database. It demonstrates a high number of core genes related to metabolic pathways intrinsic to microbial existence, such as energy metabolism (8.00%) and molecular translation (5.16%) (Figure 5). The accessory genes are related to amino acid metabolism (17.64%), carbohydrate metabolism (13.42%), and xenobiotics biodegradation and metabolism (7.51%). Most of the genes related to drug resistance are part of the accessory genome (2.24%), compared to their percentage represented in the core genome (1.89%). Similarly, genes related to infectious diseases are represented in the core genome (0.94%), accessory genome (2.24%), and strain-specific genes (2.82%).

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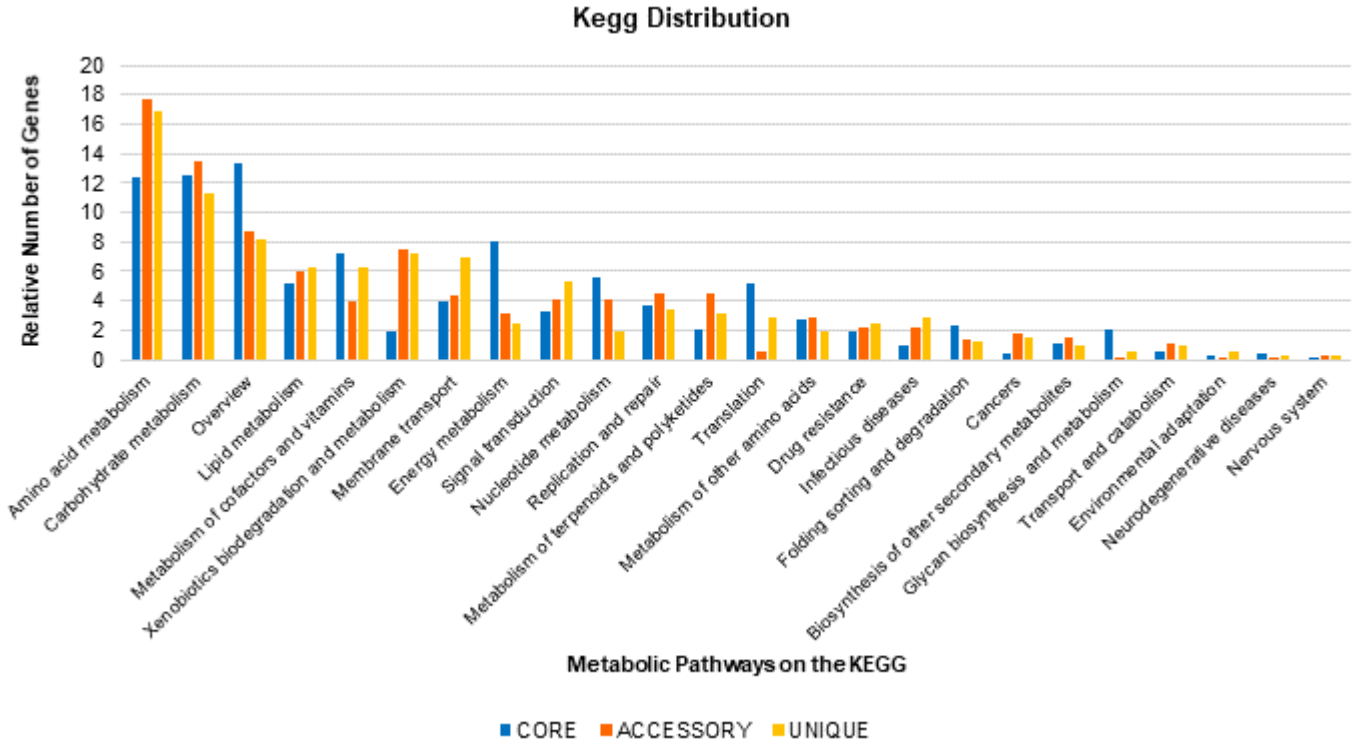

Figure 5. Graphical representation of the gene distribution by metabolic pathway within each subpartition of the total pan-genome. Only pathways with at least 0.1% of the genes represented in each subpartition of the pan-genome were considered.

As for genes related to adaptation to the environment, there is a very low gene repertoire associated with this process in the general pan-genome, with less than 1.0% of the total repertoire linked to such a pathway in any subdivision of the pan-genome.

2.5. Pan-Resistome Characterization of Acinetobacter Baumannii

A pan-resistome analysis contains analogous divisions applied to a pan-genomic analysis, but focused on microbial resistance factors [30]. Considering a similarity criterion greater than 70% and an E-value < 5×10^{-6}, all the studied strains present a pan-resistome of 171 genes, and within that, a core resistome constituted of 9 genes is shown in Table 1 [11].

Table 1. Description of the genes present in the core resistome of the studied strains containing the mechanisms of action and antibiotics associated with these mechanisms [11].

Gene	Definition	Mechanism	Antibiotic
adeK	The outer membrane factor protein in the adeIJK multidrug efflux complex	Antibiotic efflux	Phenicol, rifamycin, penem, diaminopyrimidine, tetracycline, carbapenem, macrolide, lincosamide, floroquinolone, cephalosporin
adeJ	An RND efflux protein that acts as the inner membrane transporter of the AdeIJK efflux complex	Antibiotic efflux	Diaminopyrimidine, phenicol, tetracycline, rifamycin, carbapenem, penem, fluoroquinolone, macrolide, cephalosporin, lincosamide
adeI	The membrane fusion protein of the AdeIJK multidrug efflux complex	Antibiotic efflux	Phenicol, rifamycin, penem, diaminopyrimidine, tetracycline, carbapenem, macrolide, lincosamide, floroquinolone, cephalosporin

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adeF	The membrane fusion protein of the multidrug efflux complex AdeFGH	Antibiotic efflux	Tetracycline, fluoroquinolone
adeG	The inner membrane transporter of the AdeFGH multidrug efflux complex.	Antibiotic efflux	Tetracycline, fluoroquinolone
adeL	A regulator of AdeFGH in *Acinetobacter baumannii*. AdeL mutations are associated with AdeFGH overexpression and multidrug resistance.	Antibiotic efflux	Tetracycline, fluoroquinolone
ampC	AmpC type beta-lactamases are commonly isolated from extended-spectrum cephalosporin-resistant Gram-negative bacteria.	Antibiotic inactivation	Cephalosporins
adeN	AdeN is a repressor of AdeIJK, an RND-type efflux pump in *Acinetobacter baumannii*. Its inactivation increases the expression of AdeJ.	Antibiotic efflux	Carbapenem, diaminopyrimidine, rifamycin, penem, tetracycline antibiotic, phenicol, lincosamide, fluoroquinolone, cephalosporin, macrolide
abeM	AbeM is a multidrug efflux pump found in *Acinetobacter baumannii*.	Antibiotic efflux	Acridine dye, fluoroquinolone antibiotic, triclosan

In these analyses, the strains that presented *ade*-type bombs were expected to have the complete gene repertoire to be functional. Nevertheless, this pattern was observed exclusively for the *ade*IJK efflux pump, as all the genomes presented the genes *adeI*, *adeJ*, and *adeK*. However, the same pattern was not observed for the other genes of the same family (see Supplementary Figure S3 and Supplementary Table S2). Similarly, to the genes capable of constituting the *ade*FGH pump, the presence only of the *ade*F and *ade*G genes was detected in all the strains. The gene *ade*H (the outer membrane factor protein in the *ade*FGH multidrug efflux complex) was not found in three strains (XDR-BJ83, ORAB01, and DS002), and, in theory, makes the activity of the pump unfeasible. Our study also identified an interesting protein present in all strains: the *ampC* enzyme. This is responsible for generating resistance to beta-lactams, specifically cephalosporin, and is thought to cause hydrolysis of the drug [31,32].

Analyzing the accessory portion of the resistome, an interesting distribution profile of specific genes was retrieved. The OXA-66 gene, responsible for coding a variant of beta-lactamase with action against penam, carbapenem, and cephalosporin, for example, was present in 99 strains, which is equivalent to approximately 48% of the dataset. Among these, 93 belonged to the ST 2. This fact makes this gene almost exclusive to strains belonging to ST 2. Regarding the other ST, only six strains had the OXA-66 gene, and they do not belong to ST 2, which are BAL062—ST unknown; SAA14—ST 187; XH857—ST 215; XH906—ST 922; 7847—ST unknown; TP1—ST 570.

A similar pattern was observed with the ADC-76 gene, responsible for encoding a beta-lactamase that caused cephalosporin inactivation and that was present in strains belonging exclusively to STs 23, 10, 85, 464, 575, and 639. The same was true for the OXA-68 gene, identified only in strains belonging to STs 23 and 10 but not present in all the strains. The same went for the OXA-180 gene, which was detected only in strains of ST

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267. The gene responsible for encoding OXA-69 was almost exclusive to strains belonging to STs 1, 20, 81, and 195.

Other different patterns of gene distribution can be seen in Supplementary Table S2. Nonetheless, there was no significant pattern of visible distribution related to the geographic location of the isolates, except in some cases. The OXA-67 gene was exclusive to isolates (strains EC and EH) from the Czech Republic, while the ADC-81 and OXA-92 genes were observed only in the strain A388.

Otherwise, the presence of plasmid content in *A. baumannii* is already known. Among the 206 strains selected for the study, 162 were deposited with the plasmid sequence. However, there was no statistical difference regarding the number of resistance genes in strains with plasmid versus strains that did not show plasmid (p-value = 0.3081). However, qualitative differences were expected. As an example, 21 genes were found exclusively in plasmids (see Supplementary Table S3). Among the 21 exclusive plasmid genes was the MCR-4.3 gene, the only one predicted in the entire pan-resistome with action against polymyxins.

As the distribution related to the number of antibiotics was linked to each subpartition of the pan-resistome, the antibiotic with the highest amount of resistance mechanisms linked to it was cephalosporin, with about 103 resistance proteins within the formed pan-resistome (Figure 6). In contrast, antimicrobials (sulfonamide, sulfone, cephamycin, and pleuromutilin) had low amounts of resistance mechanisms related to the predicted resistome of *A. baumannii*.

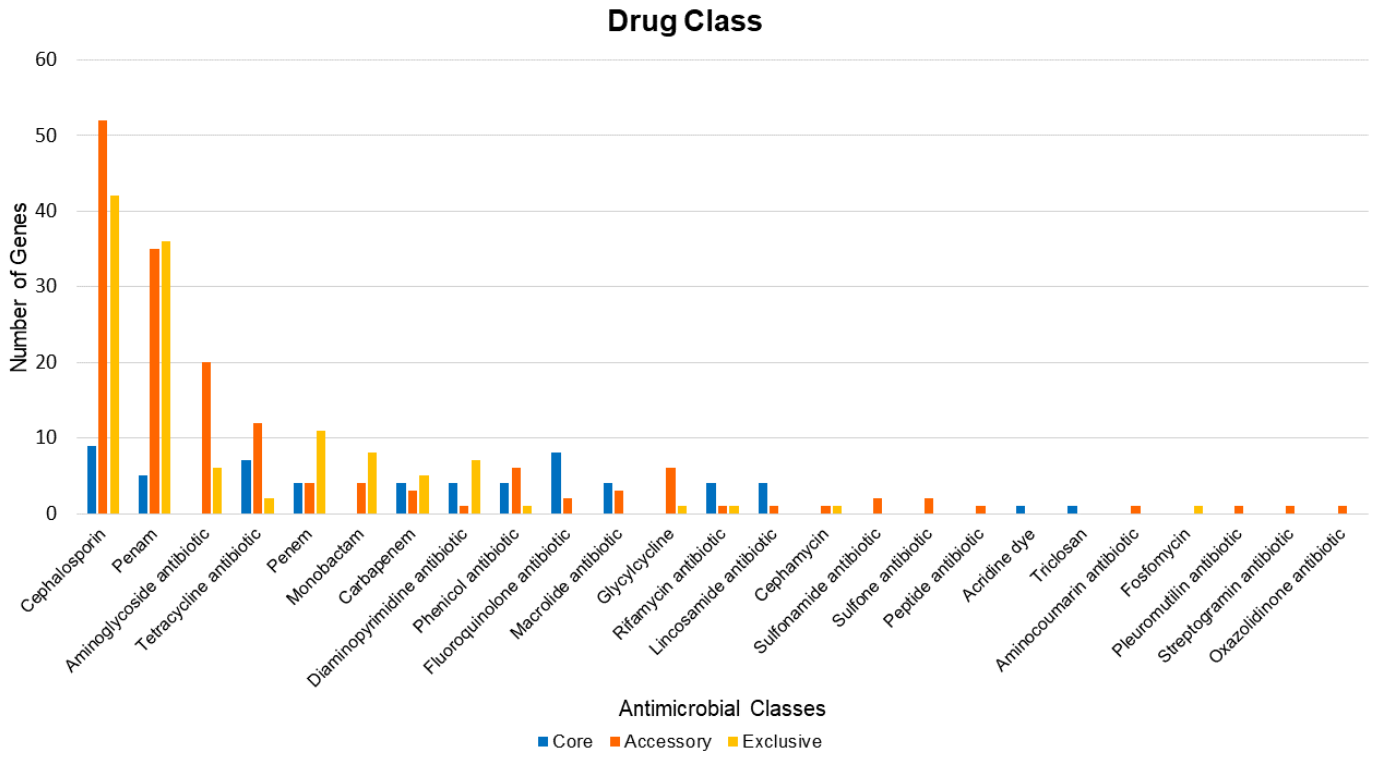

Figure 6. Distribution of the resistance mechanisms related to each antimicrobial found in the database used in the predicted total pan-resistome.

In accordance with the distribution of the types of resistance mechanisms found, 131 caused the enzymatic inactivation of the antibiotic (Figure 7). This total is equivalent to 76.6% of the predicted pan-resistome. Moreover, almost all the core resistome-related proteins are efflux pumps (8 proteins).

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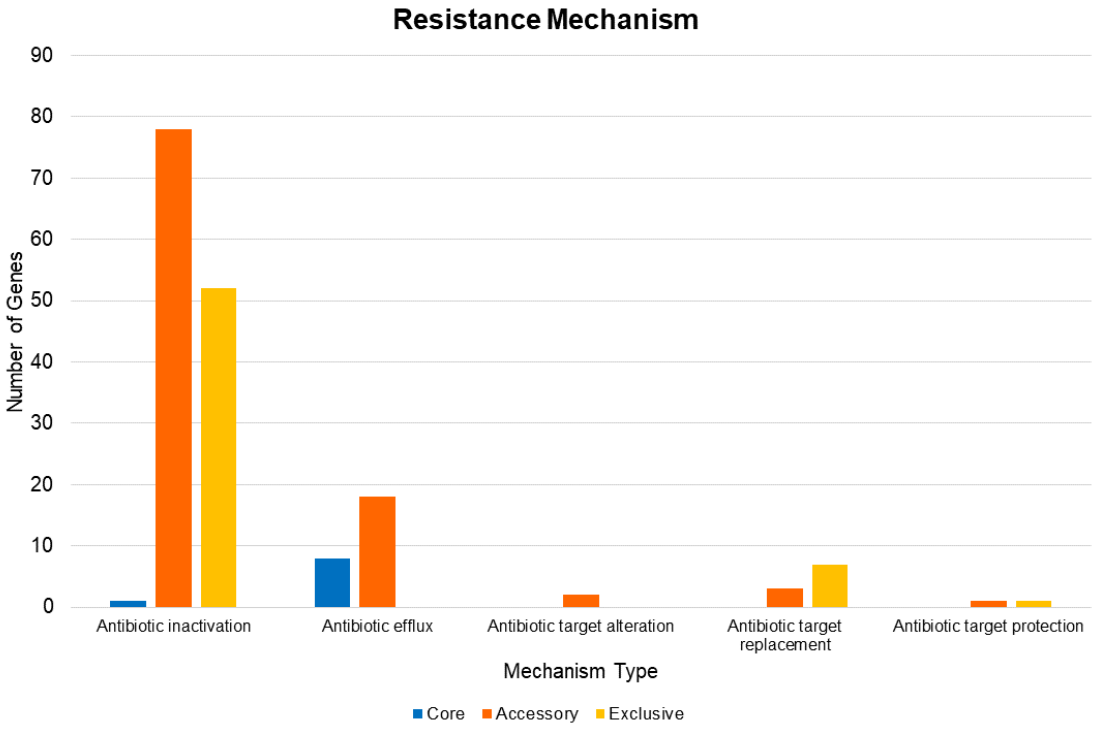

Figure 7. Distribution of the resistance mechanisms related to each type of action provided by the translated protein.

The genomics islands of resistance identified some genes, such as adeS, adeR, adeA, adeB, and adeC (within resistance island 2). Moreover, on resistance island 7 (or AbaR1 island), the following antimicrobial resistance-related genes and products were detected: sul1, qacH, AAC(3)-Ia, APH(3′)-Ia, catI, tet(A), dfrA10, ANT(3″)-IIa, OXA-10, cmlA5, arr-2, ANT(2″)-Ia, VEB-1, AAC(6′)-Ian, tet(G), floR, dfrA1, APH(6)-Id, and APH(3″)-Ib.

3. Discussion

3.1. Similarity Analysis, Geographic Distribution, and Phylogenomic Reconstruction

Recently, a more significant number of sequences of the *A. baumannii* genome provided resources for studying genomic epidemiology. Out of the 206 genomes analyzed, we identified 47 unique STs, of which STs 1, 2, 18, and 79 were distributed in significant prevalence throughout North America, Europe, and the East Asian continent, but also a diverse set of STs was indistinctly spread across the globe. The presence of few STs could be because most of the genome sequencing projects come from a single outbreak or several strains representing the same geographic location, and, in some cases, a unique ST was reported by geographic information.

According to Jeannot et al. (2014), there is a higher prevalence of strains belonging to ST 2 across the globe, but mainly in the European continent. The previous work also pointed out the polyclonality of *A. baumannii* strains within the French nation, considering the existence of different STs randomly isolated throughout the country [15].

Based on the analysis of sequence similarity of STs among the global distribution of *A. baumannii* strains, we reported several genogroups (STs) with genomic similarity less than a 98% identity in the same geographic region (Figure 1 and Supplementary Figure S1), which is opposite to the initial hypothesis that isolates from the same region exhibit high genomic similarity [23]. We also observed that the obtained result revealed a discrepancy for strains with the same ST (Figure 1 and Supplementary Figure S1). Strains from different STs in the same geographic region and the same STs isolated on other continents may maintain high similarity (>99%). In contrast, diverse genomic strains, with less than a 98% identity, are shared in nearby locations.

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In previous studies, Kazmierczak and et al. (2016) reported a heterogeneous global distribution between strains of *Pseudomonas aeruginosa* and *Enterobacter* spp. based on genetic variants of lactamases. In our study, geographically close isolates may or may not have the same variant. Consequently, phylogenetic proximity is not mandatory for members of the same geographically close species [33].

The phylogenomic tree of 206 genomes displays concordance with the ST distribution, which is expected since both analyses depend on the vertical evolutionary relationship among strains. The tree phylogeny and distribution of STs, however, show a relationship with the geographical origin. This would indicate that the dissemination of *A. baumannii* does not present a population structure. Nevertheless, it is not definitive because a higher and representative number of strains is required to evaluate the population structure.

It is biologically interesting to understand the evolution and speciation of *A. baumannii* when compared to the other species of this genus. Using this analysis, it is possible to infer the origin of specific mechanisms expressed in this species and identify the closest and the most distant members within the genus to standardize comparative analyses better. Phylogenetic analysis based on a few housekeeping genes does not represent the complete evolutionary history or the final diversity between strains or members of the same genus. Nonetheless, a study based on phylogenomics shows a refined ancestry and variety caused by changes in the niche or geographic location of bacterial populations [34].

In comparison, a previous study pointed out that, for a limited number of genes, phylogenetic inference using concatenated genes is better at portraying genetic diversity and distance between different species than the use of consensus trees derived from individual genetic analyses [35]. Therefore, through the proposed method, one can evaluate a significant distancing of *A. baumannii* strains from the other species belonging to this genus, which indicates that the use of concatenated rpoB and 16 S rRNA genes is an excellent option for the inference of phylogenetic distance of strains belonging to specific genera. A previous study obtained a similar result when performing phylogenetic inference using the complete genomes of 136 strains of *Acinetobacter* within the genus [36].

3.2. Genomic Plasticity in Acinetobacter Baumannii

Previous work has reported genomic plasticity among persistent *A. baumannii* strains in Italy [14], Argentina [37], and Australia [38]. Historically, it is a species capable of receiving and donating genes to other microorganisms in the environment, a mechanism mediated by recombination events [36].

The analysis of *A. baumannii* genomes revealed the presence of 14 essential elements of resistance within genomic islands that are acquired through the horizontal transfer of genomic recombination events [39]. The largest genomic island found (RI7) has a length of 96,878 nucleotides and presents in its content a total of 25 resistance genes, characterized by an identity of more significant than 70% against the database. This same island is partially shared by strains of different ST 1 and is more similar within strains belonging to ST 2 than ST 1. Previously, this island was described as AbaR1 resistance island, and was considered to be one of the leading genomic elements responsible for the high resistance of the species members due to its size and quantity of elements. Currently, its mobile elements are known to originate from bacteria of the genera *Pseudomonas*, *Salmonella*, and *Escherichia* [1]. Based on several studies, more than 10 islands of resistance have already been identified in the genomes of *A. baumannii* [40]. In the study of the AYE strain, seven islands of resistance were detected.

Similar events of gene displacement and the presence of specific factors related to pathogenicity within genomic islands are also reported in other species with high intrinsic resistance to antimicrobials. The presence of mobile elements containing virulence or resistance factors allows for better adaptation and proliferation of *A. baumannii*. These are usually included in some phylogenetic groups, which have greater global distribution

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[29]. Therefore, monophyletic clades have stability in gene content, which may explain its low clonal incidence compared to other clones, which are characterized by high genomic plasticity [14]. In the literature, such mechanisms are revealed of transfer and translocation in species such as *Pseudomonas aeruginosa* [41] and *Klebsiella pneumoniae* [42], microorganisms that, like *A. baumannii*, are also considered models for understanding resistomes, virulence, and pathogenicity. This fact indicates that resistance islands are persistent in the distribution of nosocomial bacteria due to selective pressure, and they are spread and fixed in bacteria to generate adaptive fitness.

3.3. Functional Characterization through Pan-Genome Analysis

Currently, pan-genome analyses, which allow for the observation of the total genetic repertoire of a species, are incredibly relevant for determining similarity, functional characterization, and analysis of exclusive characteristics of certain strains of a microbial species. Such a report aims to assess the number of genes shared by all representatives of a taxonomic set and the genes shared by more than one, but not all, strains belonging to the group, known as accessory genomes [28]. Presently, pan-genome analyses are relevant for determining genetic variability, similarity, essential genes, functional characterization, and prediction of exclusive genes by phenotypic groups to characterize species and strains, in addition to being able to also view the discrepancies between genomes that are not perceived by conventional analyses [43]. Nowadays, there are reports of pan-genome analyses of several pathogens, such as *Streptococcus agalactiae* [44], *Legionella pneumophila* [45], *Corynebacterium pseudotuberculosis* [46], *Pasteurella multocida* [47], *Pseudomonas aeruginosa* [48], and *Treponema pallidum* [49].

In the pan-genome analysis of *A. baumannii*, a core genome containing 1999 genes was identified. Biologically, using the Kyoto Encyclopedia of Genes and Genomes database, the core genome contains all the essential genes for the survival of the bacteria in a favorable environment. Therefore, it includes pathways related to metabolism and cell division, genetic processes, and energy production [28,49]. Among the genes related to the core genome, only nine are related to resistance, and these genes represent the core resistome.

On the other hand, the accessory genome has genes related to microbial adaptation mechanisms, such as antimicrobial resistance factors, symbiosis, adaptation to the environment, and virulence, which may or may not be acquired via horizontal gene transfer [50,51]. In our study, the accessory genome revealed a total of 10,337 genes that may be related to adaptation to the host and are more represented in pathways of carbohydrate and amino acid metabolism, xenobiotic metabolism, and drug resistance. Considering the pathogenic cycle of the species, xenobiotic biosynthesis and degradation pathways are essential facilitators of bacterial adaptation, mostly when related to microbial antibiosis associated with adaptation to the host [52,53], which, in theory, provides a more prolonged microbial survival. The prevalence of carbohydrate, amino acid, and xenobiotic metabolism pathways comes in part from the pathogen's evolutionary history [54,55].

In a previous study, Hassan et al. (2016) considered 30 complete genomes of *A. baumannii* for the inference of the pan-genome, reaching values of pan- and core genomes of 7606 and 2445 genes, respectively [56]. Our work suggests a more closed pan-genome (12,336), due to an increase in the core genome (1999). This comparative result was expected, considering the increase in genomes in the dataset. This fact does not invalidate the analysis made previously by the authors but it sheds light on the development of the species' pan-genome. In contrast, Mangas et al. (2019) considered 2467 complete and draft genomes of the species for inference of the pan-genome, reaching values of the core genome and pan-genome equivalent to 2221 and 19,272 respectively [57]. The result presented in this work tends to present a more closed value in relation to the core genome, which was expected since the exclusive use of complete genomes tends to increase the accuracy of orthologs' analyses. In contrast, the number of genes found in the pan-genome

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was lower than that presented by the authors. This fact can be justified by the difference in methodological approaches existing between both works.

3.4. Resistome of Acinetobacter Baumannii

In the pan-resistome analysis, it was possible to ascertain the numerical presence of a variant of beta-lactamases, such as the OXA genes. Previous studies have already pointed out that this gene is widespread among the distinct geographic locations of *A. baumannii* strains, reporting that the coding gene for OXA-143 is exclusive for Brazilian strains [58]. Moreover, the same study points out that the enzyme OXA-58 is very prevalent across the globe but has a higher incidence in strains from southeastern Europe [58]. Nevertheless, using the methodology employed, the OXA-143 gene was not detected, but a similar pattern was observed for the beta-lactamase SAT-1, which is exclusive to the Brazilian strain MRSN15313. As for the OXA-58 gene, its presence was inferred for isolated strains in Italy, India, Greece, Ghana, China, and two Mexican strains, indicating and corroborating its higher prevalence in the East.

As for the efflux pumps presented, one of the most important and studied is the *ade*ABC pump, which belongs to the RND family (resistance–nodulation–division) [9]. The same *ade*IJK pump family is adequately represented in the core resistome. Previous studies indicate that efflux pumps are excellent targets for drugs, considering that their inhibition greatly amplifies the action of antimicrobials that, under normal conditions, would be eliminated by the cell [7]. Recent studies report that inhibition of the *ade*B and *ade*J portions leads to a significant reduction in microbial resistance [59]. Both are present inside the cell and anchor the pump to the membrane. As for proteins with enzymatic action, the one that stands out the most is *ampC* beta-lactamase. It has been described with high prevalence in *A. baumannii*, which is considered one of the main species responsible for the resistance to beta-lactams [31].

Interestingly, the preferred treatment for susceptible strains of *A. baumannii* is based on carbapenems [60]; however, in evaluating the pan-resistome, many mechanisms of resistance to this class have been reported, which may suggest that its presence in the genome does not indicate expression, mainly when relating to the presence of resistance mechanisms in the genome of the SDF strain. In the case of resistant strains, tigecycline treatment has been used with varying success [10,60].

Still, in this context, it is known that the number of reports of strains resistant to polymyxins within the species has grown [61,62]. However, it was possible to predict only one gene related to the resistance phenotype to that class of antimicrobials based only on the predicted proteome of the species.

4. Materials and Methods

4.1. Genomes Database, Annotation, and Data Retrieval

All the complete *A. baumannii* genomes and their plasmids were obtained through the National Center for Biotechnology Information (NCBI)/GenBank-RefSeq [63]. An in-house Python3 script was developed to extract chromosome sequences from strains with plasmid sequences, using as a criterion the extraction of the largest contig present in the fasta file. Both files, those containing only the chromosome and those containing the chromosome and plasmid, were annotated using the same parameters in the PROKKA pipeline version 1.13.7 [64], with an additional setting: the prediction of RNAs, using RNAmmer software version 1.2 [65].

4.2. Multilocus Sequence Typing and Phylogeny

The similarity analysis was performed using all the complete genomes as input to the software FastANI [21], using default parameters. The sequence type was predicted using the MLST 2.18.0 software, based on the PubMLST platform [66]. The scheme used was the *abaumannii_2*, determined and made available by the Pasteur Institute based on seven

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sequenced housekeeping alleles: *cpn60* (Chaperonin family protein), *fusA* (Elongation factor G), *gltA* (Citrate synthase), *pyrG* (CTP synthase), *recA* (Protein RecA), *rplB* (50S ribosomal protein L2), and *rpoB* (a beta subunit of RNA polymerase) [67].

A customized Python3 script was used to extract the nucleotide sequences of 16S rRNA and rpoB genes, ranked by BLAST similarity (>98%) against *A. baumannii* AYE reference sequences. Subsequently, the extracted sequences were concatenated into a single file. The phylogeny was performed using the maximum likelihood method using the *rpoB* and 16S rRNA sequences. The alignment was performed using MAFFT software version 7.31.0 with default parameters [68], and the phylogenetic tree was inferred with the MEGA7 software [69], using the maximum likelihood method with statistical support of 10,000 bootstrap iterations to amplify the reliability of the formed clades. The generated tree figure was optimized using the FigTree 1.4.4 software [70].

4.3. Resistance Genes Profile

The Comprehensive Antibiotic Resistance Database (CARD) [11] was used to compare the local alignments and the determination of the presence of genes related to microbial resistance. For this purpose, the predicted proteome product of the automatic annotation of *A. baumannii* (206 strains) was used.

A customized Python 3.6 script was used to automate BLAST alignments [71] of proteomes against the CARD database. Only the results whose identity and coverage were equal to or greater than 70% and an E-value below 5×10^{-6}, respectively, were used. It was also used to generate the binary matrix of presence and absence genes, considering the previous mining files of the multiple alignments [72]. The final result was to generate the cluster map. The prediction of plasmid resistome was possible by comparing the annotation of the complete genomes (chromosome and plasmid) with the annotations of the chromosome only. The statistical difference related to the number of resistance factors between the strains that do not have a plasmid and those that do have a plasmid was made by the Wilcox-on-Mann–Whitney test.

4.4. Genomic Islands Analysis

Genomic Islands Prediction Software (GIPSy) [39] was used to perform the prediction of the genomic islands. In this analysis, the AYE strain was selected for the reference genome due to its history of resistance on the European continent and the high presence of resistance genes. As a subject, the genome of *A. baumannii* SDF was selected, which is a strain previously described as susceptible [1,39,73,74]. Subsequently, the BLAST Ring Image Generator (BRIG) software was used to visualize the genomic islands present in the genomes [75].

4.5. Pan-Genome and Pan-Resistome Analyses

The significant pan-genomic analyses were performed using the software Orthofinder [76]. It uses MCL (Markov Clustering algorithm) to determine the clusters of orthologous genes based on multiple alignments using the amino acid fasta as input. For the pan-genomic analysis, the annotation of both chromosomes and plasmids was considered. For the functional analysis of each subpartition, multiple comparisons against the Kyoto Encyclopedia of Genes and Genomes database (KEGG) [77] were considered. Obtaining the values related to the development of the pan-genome, as well as the alpha value was done through an in-house script.

The extracted core genome, resulting from the analysis using Orthofinder, was aligned with MAFFT [68] for subsequent phylogenomic inference using FastTree software with maximum likelihood methodology.

5. Conclusions

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There is a wide variety of genes in the total repertoire of the species studied. Unfortunately, there is no visible clustering for the host and geographic location; however, the grouping of the strains based on ST reveals a coherent pattern, corresponding to the core genome similarity. The repertoire of the resistome was characterized in terms of the presence and similarity of genes in the total pan-genome. It demonstrated enormous plasticity when evaluating the distribution of factors throughout the groups and the analyzed phylogeny. The pan-resistome also pointed out the presence of the *ade*IJK efflux pump and *amp*C enzyme in all the strains of this species, as well as the heterogeneous distribution of resistance factors across the globe. Another interesting fact is the higher amount of resistance factors to cephalosporins, aminoglycosides, and tetracycline in the studied genomes. Therefore, there is a contraindication to the use of these drugs in *A. baumannii.* These facts point mainly to the discrepancy of strains belonging to different STs within the *A. baumannii* species and its high capacity to remodel the gene repertoire to adapt to the environment or host, and, hence, can remain as an important pathogen for years. Therefore, the data collected are pertinent to better evaluate the high resistance of the species in a hospital environment and, consequently, can be used for a targeted prescription of antibiotics based on phenotyping related to a genetic presence profile. From this perspective, it is possible to use the data obtained in this work to carry out studies for new drug candidates based on the core genome and to take advantage of the assembled pan-resistome to anticipate possible escape mechanisms of *A. baumannii*.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/antibiotics10050596/s1, Figure S1: Cluster map representing the genomic similarity evaluated among all strains of Acinetobacter baumannii included in the study. The intensity of the color indicates a greater degree of similarity between the genomes. For each strain, the isolation site and the predicted sequence type were added respectively. The cladograms were generated using Euclidean distance. Figure S2: Phylogenetic tree based on the concatenated sequences of the 16S rRNA and rpoB genes representing the positioning of the Acinetobacter baumannii strains compared to the genus. Statistical support of 10,000 bootstraps was applied. The colors represent different species within the genus Acinetobacter. Figure S3: Cluster map representing the presence of resistance genes (X-axis) in all genomes (Y-axis) were addressed. In the case of existence, the color intensity represents the sequence similarity to the database used, with a minimum similarity of 70% versus the CARD database. The cladograms used are based on the Euclidean distance between the data. Table S1: Genomic data on the deposit made available by the National Center for Biotechnology Information (NCBI). The information is distributed respectively in Strain, BioSample, BioProject, Assembly, Size, GC%, and FTP for RefSeq access. Table S2: Matrix representing the pan-resistome of the strains under study. Numbers >0 represent the presence and similarity to the CARD database of the gene in the genome of the strain presented in the first column. The strains were grouped according to the phylogenomic proximity of the core genome. The first column represents the local isolation of each strain. The second column represents the sequence type predicted. Table S3: Resistance genes predicted exclusively in plasmids. The relative presence of genes considers only the 162 strains deposited with plasmid.

Author Contributions: Conceptualization, F.A., F.M.-R. and V.A.; Data curation, formal analysis and data curation, D.L.N.R., R.H. and R.G.d.S.; Writing—original draft preparation, D.L.N.R.; Writing—review and editing, F.M.-R., D.C.C., D.B., P.G., K.J.A., S.C.S., R.R., A.G.-N. and F.A.; Supervision, V.A, F.A. and F.M.-R.; Funding acquisition, V.A., F.A; Visualization, D.L.N.R. All authors have read and agreed to the published version of the manuscript.

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6. DISCUSSÃO GERAL

De acordo com a Organização Mundial da Saúde, a resistência microbiana é um problema global que pode afetar qualquer indivíduo independente de sua classe social ou região, e estima-se que em 2050 haverá uma taxa anual de 10 milhões de óbitos resultantes de infecções associadas à bactérias resistentes em todo o mundo (ESTRELA, 2018). O impacto dessa estimativa corrobora e reforça o pedido da OMS para o investimento em linhas de pesquisa relacionadas à resistência microbiana.

No que tange os resultados da metanálise desenvolvida, a chance de resistência à carbapenem apresentada por linhagens de *A. baumannii* foi significativamente maior do que a de outras bactérias. De fato, estudos anteriores de metanálise realizados com *Pseudomonas aeruginosa* apontaram que o uso constante de cabapenem na clínica médica amplia a incidência de linhagens resistentes bacterianas (VOOR IN ‘T HOLT *et al.*, 2014). É possível que um evento similar tenha ocorrido também com *A. baumannii*, o que promoveria seu atual estado multirresistente (RAMADAN *et al.*, 2018). Fato é que *P. aeruginosa* e *A. baumannii* apresentam um conjunto de similaridades resistômicas que tornam ambos modelos de estudo. É conhecido que a alta resistência à β-lactâmicos de ambas as espécies é pelo menos em parte proveniente de transferências genéticas que ocorreram entre os táxons durante e após a especiação de *A. baumannii* (HOWARD *et al.*, 2012).

No contexto genômico, *A. baumannii* apresenta uma filogenia sólida e bem resolvida. Tal fato indica alta conservação dentro da espécie, porém anteriormente o clado referente ao gênero era repleto de ambiguidades. Essa característica era observada pois *A. baumannii* era classificado juntamente com *A. nosocomialis, A. caloaceticus* e *A. pittii*, formando o complexo *Acinetobacter baumannii-calcoaceticus* (GERNER-SMIDT; TJERNBERG; URSING, 1991). Portanto, a atual classificação filogenética ocorreu devido à uma reconstrução taxonômica que segregou o antigo complexo nos clados das atuais espécies mais abundantes do gênero (SAHL *et al.*, 2013). Esse evento foi extremamente importante pois os microrganismos de maior importância clínica do gênero são respectivamente *A. baumannii* e *A. nosocomialis*, e sua classificação errônea poderia desencadear erros em cascata em estudos como o aqui apresentado, enviesando análises multi-omicas.

Com relação à análise pangenômica, a relação entre as linhagens da espécie é concisa havendo pouca conservação genética quando comparada a análise de microrganismos extremantes clonais como *Corynebacterium pseudotuberculosis* (SOARES *et al.*, 2013). Todavia, esse tipo de relação é observada em outros patógenos Gram-negativos de

comportamento similar à *A. baumannii*, como *P. aeruginosa* que apresenta um genoma central equivalente à cerca de 1% do pangenoma total (FRESCHI *et al.*, 2019). *Klebsiella pneumoniae*, outro patógeno pertencente ao grupo ESKAPE, apresentou também um resultado similar, com um valor de genoma central equivalente à 1.743 genes e um pangenoma constituído por 29.886 genes (HOLT *et al.*, 2015). Tais fatos podem estar relacionados então à alta adaptação dessas espécies ao hospedeiro humano e ao ambiente hospitalar, o que implica à exposição constante ao estresse ambiental, forçando o genoma a manter uma alta plasticidade para evitar a eliminação da espécie ao longo do tempo (HOWARD *et al.*, 2012; SCHEUERL *et al.*, 2020; SHEPPARD; GUTTMAN; FITZGERALD, 2018). Infelizmente o pangenoma da espécie permanece aberto, o que indica que novos genes podem ser adicionados ao repertório total. Tal fato significa que o desenvolvimento da história de resistência da espécie tende a ser imprevisível, pois não se sabe ao certo quais mecanismos podem ser adquiridos ou alterados ao longo do tempo.

Observamos ainda que *A. baumannii* apresenta alta plasticidade genômica relacionada à presença de ilhas genômicas diretamente ligadas aos seus principais fatores de resistência. Anteriormente havia sido descrito na literatura que diversos elementos que constituem o repertório de mecanismos de resistência da espécie são de fato provenientes de outros patógenos, sendo: *Pseudomonas spp.*, *Salmonella spp.* e *Escherichia spp.* (FOURNIER, Pierre-Edouard *et al.*, 2006). Sabe-se ainda que parte dos mecanismos de resistência de *A. baumannii* são relacionados ao plasmídeo. Nesse trabalho, desvendamos que não há diferença estatística entre a quantidade de genes de resistência presentes em linhagens que possuem plasmídeo e linhagens que não possuem plasmídeos. Todavia, foi possível observar a formação de um perfil de 23 genes encontrados exclusivamente em plasmídeos, dentre eles o único gene relacionado a resistência à polimixinas (gene MCR-4.3). A maior prevalência desse gene em plasmídeos foi observada anteriormente na espécie (ALCOCK *et al.*, 2020; MARTINS-SORENSON *et al.*, 2020).

Com base no resistoma levantado e observando o comportamento da espécie alvo desse trabalho, esperava-se que com a análise *in silico* das 206 linhagens da espécie fosse possível observar um padrão de agrupamento genético de acordo com o local de isolamento, porém não foi possível chegar à tal conclusão. Contudo, é possível observar um padrão genômico que segue a distribuição por Sequência Tipo (ST), o que leva à hipótese de que linhagens pertencentes ao mesmo grupo de tipagem tendem a possuir mecanismos de resistência similares, mesmo não compartilhando o mesmo local de isolamento geográfico. Todavia, não existe um padrão resistômico claro o suficiente para determinar um método diagnóstico e

epidemiológico de análise de resistência baseado exclusivamente em ST. Um fato similar foi observado em *K. pneumoniae* durante estudos que consideraram apenas regiões dos Estados Unidos da América (CERQUEIRA *et al.*, 2017). Fato é que microrganismos se deslocam facilmente junto com as grandes massas populacionais e, portanto, uma distribuição homogênea ou equivalente ao redor do globo também não é inesperada.

Por fim, a análise do resistoma de *A. baumannii* indica que esse patógeno possui um repertório robusto e complexo, especialmente voltado à resistência a β-lactâmicos, aminoglicosídeos e tetraciclinas, o que implica em uma maior dificuldade na busca de fármacos preferenciais para tratamentos. Do mesmo modo, houve baixa predição de mecanismos de resistência à gliciclinas e polimixinas, o que corrobora com a escolha dessas classes como alternativas farmacológicas, porém ainda assim existem linhagens já resistentes a ambas as drogas (GARCIA CASALLAS *et al.*, 2019; HUTTNER *et al.*, 2012; NAVON-VENEZIA; LEAVITT; CARMELI, 2007; SNYMAN *et al.*, 2020).

7. CONCLUSÕES GERAIS

Considerando os dados apresentados, é possível concluir que:

- *A. baumannii* apresenta chance de resistência à carbapenem superior à de outras bactérias, o que corrobora com seu histórico como patógeno levantado e exaltado pela OMS;
- O perfil de genes de resistência da espécie tende a manter um agrupamento por sequência tipo. Além disso, há um grande número de mecanismos voltados para a resistência à β-lactâmicos, aminoglicosídeos e tetraciclinas, o que justifica a preocupação dos órgãos de saúde globais para com esse microrganismo;
- A espécie apresenta-se em um clado monofilético dentro do gênero, o que dificulta o enviesamento de análises genômicas comparativas;
- Filogenomicamente, os clados da espécie apresentam agrupamentos baseados em sequência tipo, e há alta conservação genômica dentro desses clados;
- O genoma central da espécie é conciso e seu pangenoma é robusto, permanecendo aberto, fatos esses associados à alta plasticidade genômica observada;
- O entendimento dos mecanismos de resistência relacionados à espécie estudada, também auxilia no entendimento de mecanismos de resistência de espécies correlatas do grupo ESKAPE estudadas por outros grupos de pesquisa;
- Espera-se que os dados desse trabalho possam ser utilizados como base para estudos estruturais que visem a busca por novas estratégias de combate a infecções causadas por *A. baumannii*.

8. PERSPECTIVAS

As principais perspectivas deste trabalho são:

- Analisar a diversidade das linhagens de *A. baumannii* ao redor do globo à nível de *indels* e polimorfismos de nucleotídeos únicos;
- Realizar análise pangenômica por Sequência Tipo, afim de determinar a presença de subgrupos;
- Aplicar a abordagem deste estudo em outros patógenos de importância clínica mundial, como *Pseudomonas aeruginosa*;
- Aplicar a metodologia de vacinologia reversa para adquirir novos candidatos vacinais e alvos para drogas contra *A. baumannii*, como é preconizado pela OMS.

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10. ANEXOS

9.1 ATIVIDADES ACADÊMICAS

Durante o decorrer do processo de mestrado foram desenvolvidas atividades acadêmicas, as quais estão citadas no texto a seguir referente ao currículo *lattes*.

- Um (1) artigo publicado como primeiro autor:

RODRIGUES, D. L. N.; MORAIS-RODRIGUES, F.; HURTADO, R.; SANTOS, R. G.; COSTA, D. C.; BARH, DEBMALYA; GHOSH, PREETAM; ALZAHRANI, K. J.; SOARES, S. C.; RAMOS, R.; GOES NETO, A.; AZEVEDO, V. A. C.; ABURJAILE, F. F. Pan-resistome insights into the multidrug resistance of *Acinetobacter baumannii*. **ANTIBIOTICS-BASEL**, v. 10, n. 5, p. 596–615, 18 maio 2021. DOI: https://doi.org/10.3390/antibiotics10050596

- Um (1) artigo publicado com primeira autoria compartilhada:

MORAIS RODRIGUES DA COSTA, FRANCIELLY*; **LUCAS NERES RODRIGUES, DIEGO***; SILVÉRIO-MACHADO, RITA; GABRIEL RODRIGUES GOMES, LUCAS; BENTES KATO, RODRIGO; GONÇALVES DOS SANTOS, ROSELANE; A. C. AZEVEDO, VASCO; A DOS SANTOS, MARCOS. A Genes selected after application modified logistic regression in the microarrays gene expression for breast cancer. **International Journal of Scientific Research and Management**, v. 8, n. 12, p. 85–95, 27 dez. 2020.

- Um (1) artigo publicado como co-autor:

MORAIS-RODRIGUES, FRANCIELLY; SILV'ERIO-MACHADO, RITA; KATO, RODRIGO BENTES; **RODRIGUES, DIEGO LUCAS NERES**; VALDEZ-BAEZ, JUAN; FONSECA, VAGNER; SAN, EMMANUEL JAMES; GOMES, LUCAS GABRIEL RODRIGUES; DOS SANTOS, ROSELANE GONÇALVES; VINICIUS CANÁRIO VIANA, MARCUS; DA CRUZ FERRAZ DUTRA, JOYCE; TEIXEIRA DORNELLES PARISE, MARIANA; PARISE, DOGLAS; CAMPOS, FREDERICO F; DE SOUZA, SANDRO J; ORTEGA, JOSÉ MIGUEL; BARH, DEBMALYA; GHOSH, PREETAM; AZEVEDO, VASCO A.C.; DOS SANTOS, MARCOS A. Analysis of the microarray gene expression for breast cancer progression after the application modified logistic regression. **Gene**, v. 726, p. 144168, 5 fev. 2020.

- Um (1) capítulo de livro publicado como co-autor:

RODOVALHO, V. de R.; **RODRIGUES, D. L. N.**; JAN, G.; LOIR, Y. L.; AZEVEDO, V. A. de C.; GUÉDON, E. *Propionibacterium freudenreichii*: General Characteristics and Probiotic

Traits. [S. l.]: IntechOpen, 2021. DOI 10.5772/intechopen.97560. Available at: https://www.intechopen.com/online-first/propionibacterium-freudenreichii-general-characteristics-and-probiotic-traits. DOI: http://dx.doi.org/10.5772/intechopen.97560

- Um (1) resumo expandido publicado em anais de evento:

RODRIGUES, D. L. N.; MORAIS-RODRIGUES, F.; SANTOS, R. G.; ABURJAILE, F. F.; AZEVEDO, V. A. C. ANÁLISE FUNCIONAL DO PANGENOMA DE *Acinetobacter baumannii*. In: **VII Simpósio de Microbiologia da UFMG** - Conecta SIM, 2020, Belo Horizonte. Simpósio de Microbiologia da UFMG, 2020.

- Nove (9) apresentações de trabalhos em eventos:

RODRIGUES, D. L. N.; MORAIS-RODRIGUES, F.; SANTOS, R. G.; ABURJAILE, F. F.; AZEVEDO, V. A. C. Resistome Profile of *Acinetobacter baumannii*, 2020. (Congresso). **X-meeting eXperience 2020**.

VALDEZ-BAEZ, JUAN; HURTADO, R. E. C.; **RODRIGUES, D. L. N**.; RODOVALHO, V. R.; MALCHER, F. M.; SANTOS, R. G.; CERQUEIRA, J. C.; KATO, R. B.; GOMIDE, A. C. P.; MORAIS-RODRIGUES, F.; AZEVEDO, VASCO A.C. Accessory genome provides insights of potential probiotic and classification of *Enterococcus faecium* strains, 2019. (Conferência ou palestra) **2 Associated International Laboratory Meeting (LIA 2019) - Bact-Inflam Conference**.

VALDEZ-BAEZ, J. L.; **RODRIGUES, D. L. N**.; GOMIDE, A. C. P.; KATO, R. B.; GALA-GARCIA, A.; MORAIS-RODRIGUES, F.; AZEVEDO, VASCO A.C. ANOTAÇÃO FUNCIONAL DO GENOMA REVELA O POTENCIAL PROBIOTICO DE Bacillus clausii DSM8716, 2019. (Simpósio) **VI Simpósio de Microbiologia da UFMG – CONECTA SIM**.

RODRIGUES, D. L. N.; VALDEZ-BAEZ, J. L.; SANTOS, R. G.; SILVA, A. L.; TOSTA, S. F. O.; HURTADO, R. E. C.; GALA-GARCIA, A.; GOMIDE, A.; KATO, R. B.; CERQUEIRA, J. C.; MORAIS-RODRIGUES, F.; AZEVEDO, VASCO A.C. Brief Comparative Genomics about Lactobacillus *casei*, 2019. (Congresso) **2 Associated International Laboratory Meeting (LIA 2019) - Bact-Inflam Conference**.

RODRIGUES, D. L. N.; VALDEZ-BAEZ, J. L.; SANTOS, R. G.; TOSTA, S. F. O.; SILVA, A. L.; GOMIDE, A. C. P.; KATO, R. B.; GALA-GARCIA, A.; MORAIS-RODRIGUES, F.; AZEVEDO, VASCO A.C. COMPARAÇÃO FILOGENÔMICA DE SEIS LINHAGENS E CLASSIFICAÇÃO DENTRO DO GÊNERO DE *Lactobacillus casei*, 2019. (Simpósio) **VI Simpósio de Microbiologia da UFMG – CONECTA SIM**.

KATO, R. B.; **RODRIGUES, D. L. N.**; VALDEZ-BAEZ, J. L.; SANTOS, R. G.; TOSTA, S. F. O.; SILVA, A. L.; GOMIDE, A. C. P.; GALA-GARCIA, A.; MORAIS-RODRIGUES, F.; AZEVEDO, V. A. C. Comparative genomics analysis and classification of the *Lactobacillus casei* species, 2019. (Congresso). **X-Meeting 2019 - 15th Internacional Conference of the Brazilian Association of Bioinformatics and Computational Biology (AB3C)**.

RODRIGUES, D. L. N.; HURTADO, R. E. C.; COSTA, D. C.; GOMIDE, A. C. P.; AZEVEDO, V. A. C.; MORAIS-RODRIGUES, F.; ABURJAILE, F. F. Comparative genomics of *Acinetobacter baumannii* strains, 2019. (Congresso) **X-Meeting 2019 - 15th Internacional Conference of the Brazilian Association of Bioinformatics and Computational Biology (AB3C)**.

RODRIGUES, D. L. N.; AZEVEDO, VASCO A.C. Genômica comparativa de diferentes linhagens de *Lactobacillus casei*, 2019. (Comunicação) **I Comunicando Ciência**.

CERQUEIRA, J. C.; SANTOS, R. P. S.; SILVA, A. L.; HURTADO, R. E. C.; ALMEIDA, M. O.; SOUZA, T. J.; **RODRIGUES, D. L. N.**; VALDEZ-BAEZ, J. L.; MORAIS-RODRIGUES, F.; GOMIDE, A. C. P.; FIGUEIREDO, H.; WATTAM, A. R.; SILVA, A.; AZEVEDO, V. A. C.; VIANA, M. V. C. Taxonomy and comparative genomics of Corynebacterium ulcerans strain isolated from Pig, previously identified as *C. pseudotuberculosis*, 2019. (Congresso) **X-Meeting 2019 - 15th Internacional Conference of the Brazilian Association of Bioinformatics and Computational Biology (AB3C)**.

- Dois (2) cursos de curta duração ministrados:

DOS SANTOS, ROSELANE GONÇALVES; **RODRIGUES, DIEGO LUCAS NERES** Genômica Comparativa, 2020. (Extensão) Palavras-chave: **Genomica comparativa**, Bioinformática, Biologia computacional Áreas do conhecimento: Biologia Geral,Genética Molecular e de Microorganismos, Biologia Molecular. 2 horas.

MORAIS-RODRIGUES, F.; GOMIDE, A. C. P.; SILVA, A. L.; SANTOS, R. G.; **RODRIGUES, D. L. N**.; AZEVEDO, VASCO A.C. **Treinamento de Montagem, Anotação e Depósito de genomas de procarioto**, 2020. (Aperfeiçoamento) Palavras-chave: Bioinformática, Biologia computacional, Procariotos, Montagem de genomas Áreas do conhecimento: Genética Molecular e de Microorganismos, Linguagens de Programação. 80 horas.

- Um (1) programa de computador registrado:

PanViTa - Pan Virulence and resisTance Analysis, 2021, Brasil. Instituição de Registro: UFMG - Universidade Federal de Minas Gerais, **Número do Registro: 20210006**. Data de depósito: 25/01/2021, Data da concessão: 25/01/2021. Instituição(ões) Financiadora(s): FAPEMIG; CNPq; CAPES. Link: https://github.com/dlnrodrigues/panvita

- Três (3) organizações de eventos e/ou monitorias:

GOES NETO, A.; A. C. AZEVEDO, VASCO; ABURJAILE, F. F.; JAISWAL, A. K.; SOARES, S. C.; PARISE, M.; PARISE, D.; REZENDE, D.; RODRIGUES, D. L. N. **Curso de Filogenia: pequena-escala (Filogenética) e larga-escala (Filogenômica)**, 2020. (Outro, Organização de evento).

KATO, R. B.; FRANCO, G. R.; RODRIGUES, D. L. N. **IV Curso de Versão em Bioinformática** UFMG, 2020. (Outro, Organização de evento).

AZEVEDO, VASCO A.C.; RODRIGUES, D. L. N. **2 Associated International Laboratory Meeting (LIA 2019) - Bact-Inflam Conference**, 2019. (Congresso, Organização de evento).

- Onze (11) eventos como ouvinte:

49ª Reunião Anual da Sociedade Brasileira de Bioquímica, 2020. (Seminário).

I Workshop Online de Bioinformática (WOB20), 2020. (Oficina)

Seminários de Bioinformática 2020, 2020. (Seminário)

VII Simpósio de Microbiologia da UFMG - Conecta SIM, 2020. (Simpósio)

Workshop de Avaliação Discente, 2020. (Feira)

X Workshop de Genética, Conservação e Biologia Evolutiva, 2020. (Oficina)

X-meeting eXperience 2020, 2020. (Congresso)

2 Associated International Laboratory Meeting (LIA 2019) - Bact-Inflam Conference, 2019. (Congresso)

ANIMAL SEX DETERMINATION BY GENES, CHROMOSOMES AND THE ENVIRONMENT, 2019. (Seminário)

VI Simpósio de Microbiologia da UFMG – CONECTA SIM: Microbiologia Interligada, 2019. (Simpósio)

X-Meeting 2019 - 15th Internacional Conference of the Brazilian Association of Bioinformatics and Computational Biology (AB3C), 2019. (Congresso)