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Predicting carbapenemase production in Pseudomonas aeruginosa using machine learning on disk diffusion data

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Article

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Abstract

Purpose: Detection of carbapenemase-production in *Pseudomonas aeruginosa* is challenging due to the co-occurrence of multiple resistance mechanisms. We developed a novel machine learning approach to predict carbapenemase production based on inhibition zone diameters (IZD) from routine disk diffusion testing.

Methods: A CatBoost model (CBM) was trained on 246 whole-genome-sequenced clinical *P. aeruginosa* isolates, including 95 carbapenemase-producing *P. aeruginosa* (CPPA). Models based on different antibiotic combinations were evaluated and externally validated on two independent datasets. Performance was benchmarked against the phenotypic screening algorithm described by Gill *et al.*

Results: For Dataset 1, CBM achieved a sensitivity and specificity of 94% and 98%, compared with 94% and 38% for the Gill *et al.* algorithm. For Dataset 2, sensitivity/specificity were 95%/98% for CBM vs 100%/0% for the Gill algorithm. This represented a reduction in isolates requiring confirmatory testing of 76% in Dataset 1 and 52% in Dataset 2.

Conclusions: CatBoost models trained on IZD represent a valuable tool for carbapenemase prediction in PA, substantially improving diagnostic performance and reducing the number of confirmatory tests required. These findings highlight the potential of machine learning to streamline the detection of resistance mechanisms within routine susceptibility testing workflows and may improve recognition of CPPA, especially in resource-limited settings.

Importance: This approach could reduce unnecessary confirmation-testing for carbapenemases in *P. aeruginosa*, saving time and resources in clinical settings. It underscores the value of integrating machine learning with phenotypic data to address antimicrobial resistance challenges.

Introduction

Infections due to *Pseudomonas aeruginosa* (PA) are a major challenge in patient care, as they are associated with severe and often life-threatening diseases. Diagnostics and treatment are often complicated by the ability of PA to combine multiple resistance mechanisms like porin loss (e.g., OprD deficiency) (1, 2), overexpression of efflux pumps (e.g., MexAB-OprM) (3), activation of intrinsic β-lactamases including AmpC and oxacillinases, as well as the acquisition of extended-spectrum β-lactamases (ESBLs) (4) and carbapenemases, predominantly Ambler class B metallo-β-lactamases such as VIM-2 and NDM-1 (5–8). In particular, carbapenem-resistant PA severely limits available therapeutic options and has therefore been classified by the World Health Organization as high-priority pathogen (9).

Because of the frequent co-occurrence of various resistance mechanisms the detection of carbapenemase production in PA remains challenging (7). Recommendations of the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) for testing of carbapenemase production in PA focus on infection prevention, local outbreak investigations and surveillance (10, 11). Recently, the Infectious Diseases Society of America (IDSA) has issued a recommendation for the testing of difficult-to-treat resistant (DTR) PA with possible therapeutic relevance, especially the distinction between serine- and MBL carbapenemases, as this could influence the empirical therapy (12). Confirmation of carbapenemase production generally requires additional testing, using phenotypic assays (e.g. mzCIM), lateral flow immunoassays, or molecular methods, such as polymerase chain reaction (PCR) or whole-genome sequencing (WGS). However, such tests are often not available in routine microbiology laboratories and low resource settings and are associated with high cost, increased technical complexity, and longer turnaround times. Therefore, accurate pre-classification of carbapenemase-negative isolates is essential to avoid unnecessary confirmatory testing. In Enterobacterales, EUCAST recommends screening cutoffs based on meropenem and ertapenem susceptibility, including reduced inhibition zone diameters or elevated minimum inhibitory concentration (MIC) values (10). Isolates meeting these thresholds should undergo additional testing for carbapenemase production. In routine diagnostics, this process can be simplified as a decision tree: depending on predefined criteria (ertapenem/meropenem MIC values or inhibition zone diameters), the diagnostic algorithm proceeds toward the suspicion or exclusion of a carbapenemase.

Recently, machine-learning approaches, particularly Random Forest models trained on disk diffusion inhibition zone diameters, have demonstrated superior sensitivity and specificity compared with conventional screening cutoffs for Enterobacterales (13, 14). An alternative machine learning method known as gradient boosting uses a different approach. Instead of constructing multiple decision trees in parallel, gradient boosting relies on a sequential building process in which each subsequent tree is specifically designed to correct the errors of its predecessor (Fig. 1). A modern implementation of this approach is CatBoost. It has proven to be both powerful and robust when applied to tabular clinical data (15).

These models provide a promising strategy for predicting carbapenemase production solely on the basis of antimicrobial susceptibility testing data, thereby reducing the number of isolates requiring confirmatory testing without overlooking carbapenemase-producing strains.

In contrast to Enterobacterales, no reliable algorithm for the prediction of carbapenemase production in PA is available, which is recommended by CLSI or EUCAST. Nevertheless, several antibiotic susceptibility-based approaches have been proposed. Gill *et al.* demonstrated such an approach in a prospective multicentre study, based on resistance to meropenem or imipenem and to ceftazidime and cefepime, or, as an additional criterion, resistance to meropenem or imipenem and non-susceptibility to ceftolozane-tazobactam (16) (Fig. 1).

The aim of this study was to evaluate whether a CatBoost model based on inhibition zone diameters can reliably predict carbapenemase production in PA while reducing unnecessary confirmatory testing.

Materials and Methods

Study design

In this study, we trained a CatBoost model (CBM) with inhibition zone diameters (in mm) of four different antibiotic panels (standard panel, novel β-lactam panel, complete panel and minimal panel; see Fig. 2) on the internal training dataset. The trained models were evaluated on two external datasets, with sensitivity, specificity, area under the receiver operating characteristic curve (AUROC), Youden index and reduction in confirmatory testing as performance metrics (Fig. 2).

For comparison, the phenotypic algorithm as described by Gill *et al.* was applied to the corresponding datasets (16).

Model parameters

We trained CatBoost models using the optimized hyperparameters put forward by RealMLP (17). The model uses a moderate depth of 7 trees, a 0.08 learning rate, and up to 1,000 boosting iterations. It applies mild stochastic regularization through 90% Bernoulli subsampling and a slightly below-default split-score randomization strength of 0.8, with early stopping after 300 rounds.

Description of training- and external datasets

The CBMs were trained on an internal dataset comprising 246 non-duplicate PA isolates provided by the German National Reference Centre for Multidrug-resistant Gram-negative bacteria (NRC) and the University Institute for Medical Microbiology and Virology, Oldenburg, Germany (Centre 1). Of these 246 isolates, 95 were carbapenemase-positive (CPPA) and 151 were carbapenemase-negative (nCPPA). Inclusion-criteria for nCPPA in the internal dataset was resistance to imipenem (n = 137) and/or meropenem (n = 124), additional resistance to ceftazidime was present in 57 isolates. One isolate harboured bla_{KPC-2}, while all other carbapenemase-positive isolates carried metallo-β-lactamase genes including bla_{VIM-2} (n = 50), bla_{NDM-1} (n = 13), and bla_{GIM-1} (n = 13) (Table S1). Two isolates harboured two carbapenemase genes: bla_{IMP-1} and bla_{DIM-1} (NRC); and bla_{GES-5} and bla_{NDM-1}. Detailed isolate characteristics and carbapenemase distribution are provided in Table S2 and Figure S1.

For a comprehensive assessment of model performance, two external datasets were used. Dataset 1, originating from SYNLAB MVZ Leverkusen (Centre 2), Germany, comprised 56 nCPPA and 17 CPPA. bla_{VIM-2} was the most prevalent carbapenemase (n = 8), followed by bla_{NDM-1} (n = 4), bla_{GIM-1} (n = 2), bla_{FIM-1} (n = 1), and bla_{VIM-1} (n = 1). In addition, one isolate harboured two carbapenemases, bla_{NDM-1} and bla_{VIM-4} (n = 1). The negative control group comprised isolates resistant to meropenem (n = 49), imipenem (n = 56) and ceftazidime (n = 49). All isolates from the training dataset and dataset 1 underwent whole genome sequencing for the detection of carbapenemase genes, as previously described (18). Dataset 2, originating from the University of Zurich, Switzerland (Centre 3), comprised 136 isolates, of which 71 were CPPA and 65 were nCPPA (19, 20). bla_{VIM-2} was the most prevalent enzyme (n = 16), followed by bla_{IMP-1} (n = 14), other VIM variants ($bla_{VIM-1, -4, -5, -6, -11, -28}$; n = 21), IMP variants ($bla_{IMP-7, -15, -18}$; n = 3), and bla_{NDM-1} (n = 8), with single occurrences of bla_{AIM-1}, bla_{SPM-1} and bla_{GES-5}. Five isolates co-produced two carbapenemases: bla_{NDM-1} / bla_{GES-5} (n = 1), $bla_{NDM-1/IMP-1}$ (n = 2), $bla_{OXA-181/IMP-26}$ (n = 1) and $bla_{NDM-1/VIM-2}$ (n = 1). The nCPPA group consisted of 65 non-carbapenemase-producing isolates, of which 65 were resistant to ceftazidime, 65 to imipenem, and 55 to meropenem.

Table S5 provides an overview of antibiotic resistance for selected antibiotics. Detailed descriptions of these two validation datasets are provided in Tables S3, S4 and Figure S2-S3.

Susceptibility testing

Antimicrobial susceptibility testing for the internal dataset and dataset 1 was performed at the Institute of Medical Microbiology and Virology, University Oldenburg using disk diffusion testing according to EUCAST standards (21). Antibiotic disks included piperacillin, piperacillin-tazobactam, ceftazidime, ceftazidime-avibactam, ceftolozane-tazobactam, cefepime, cefiderocol, cefepime-enmetazobactam, aztreonam, aztreonam-avibactam, imipenem, meropenem, imipenem-relebactam (Liofilchem, Roseto degli Abruzzi, Italy) as well as meropenem-vaborbactam, ciprofloxacin and amikacin (Oxoid, Basingstoke, UK). Susceptibility data for dataset 2 were provided by Mancini *et al.* and included the antibiotics mentioned above, with the exception of piperacillin and aztreonam-avibactam (19).

Phenotypic Algorithm

The phenotypic algorithm was applied as described by Gill *et al.* (16). Briefly, the screen was classified as positive when the EUCAST categorical interpretation indicated resistance to at least imipenem or meropenem (OR condition) in combination with non-susceptibility to both ceftazidime and cefepime (AND conditions). If the primary screen was negative—i.e., the OR condition was not met or at least one of the AND conditions were not fulfilled—a secondary criterion was applied: resistance to imipenem and/or meropenem combined with resistance to ceftolozane-tazobactam. If this secondary criterion was also not met, the screen was classified as negative. The EUCAST "I" category was classified as susceptible (Fig. 1).

Training of the CatBoost model

To train the CatBoost model (CBM), IZD of the internal training dataset were first used (Fig. 2). We optimized binary cross-entropy and performed model selection on the validation set (based on AUROC scores) to prevent overfitting. To identify the most suitable modelling approach, different combinations of antibiotics were evaluated based on validation set scores. Initially, the model was trained using inhibition zone diameters derived from the “standard” disk diffusion panel routinely applied for *P. aeruginosa* susceptibility testing. This panel comprised piperacillin, piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, ciprofloxacin, and amikacin.

In a second step, inhibition zone diameters of the “novel β-lactam panel”, consisting of ceftazidime-avibactam, ceftolozane-tazobactam, cefiderocol, cefepime-enmetazobactam, aztreonam-avibactam, imipenem-relebactam, and meropenem-vaborbactam were used exclusively.

As a next step, the performance of a combined approach incorporating inhibition zone diameters from both the standard- and novel β-lactam panels was evaluated (“complete panel”). In order to increase the usability of the model, antibiotic combinations were identified that reduced the total number of antibiotics required but did not lead to a significant loss of sensitivity and specificity. This resulted in a 6-antibiotic profile “minimal-panel”, consisting of cefepime, ceftolozane-tazobactam, imipenem, meropenem, aztreonam and imipenem-relebactam.

The CBM was optimized to distinguish carbapenemase-producing *P. aeruginosa* from isolates with carbapenem resistance mediated by non-carbapenemase mechanisms, with particular emphasis on high sensitivity while maintaining sufficient specificity to reduce unnecessary confirmatory testing. A target sensitivity of at least 95% was predefined for the different antibiotic panels.

External validation

The classification thresholds (i.e., the probability cutoff at which the model classifies an isolate as CPPA) were determined on the internal training set. The CBMs were then applied to the external validation datasets (dataset 1 and dataset 2). Notably, two antibiotics (aztreonam-avibactam and piperacillin) were not available in dataset 2, thereby demonstrating the models' inherent robustness to missing values.

Webtool

The CBM trained in this study was integrated into the web application „CarbaDetector”, which was developed for carbapenemase detection in Enterobacterales. The new version „CarbaDetector Pseudomonas“ is accessible at https://uol.de/carba-detector (14). Users may enter inhibition zone diameters obtained by disk diffusion testing. Based on these input values, the web tool selects the most suitable model and corresponding antibiotic panel and predicts the probability of carbapenemase production. Generative artificial intelligence (ChatGPT, GPT-5.6 Sol; OpenAI) was used as a coding assistant to support the integration of the trained CatBoost models into the existing R/Shiny-based CarbaDetector web application. All AI-assisted code was reviewed and validated by the authors.

Statistical analysis

For the machine-learning models, sensitivity, specificity, area under the receiver operating characteristic curve (AUROC), and corresponding 95% confidence intervals (CIs) based on bootstrapping on the test set (n = 1000 iterations) were calculated using Python. The Youden index for all models was calculated using Microsoft Excel (Version 16.113.1). For the Gill algorithm, sensitivity, specificity, and corresponding 95% CIs were also calculated using Microsoft Excel, with 95% CIs for sensitivity and specificity determined using the Wilson score method. The reduction in confirmatory testing was calculated relative to confirmatory testing of all isolates as $[1 - (TP + FP)/N] \times 100$.

Inhibition zone diameter (IZD) distributions were visualized using histograms generated in R-Studio (version 4.3.1) using the ggplot2 (v4.0.2) package. Schematic illustrations were created with BioRender (BioRender.com).

Feature importance

We quantified the relevance of input features by means of Shapley values as obtained by the SHAP treeexplainer (version 0.46.0) (22). These provided local attributions for every sample in the test set, which could be summarized using Beeswarm plots. We quantified the global importance of features using mean absolute Shapley values.

Results

Performance on the internal test dataset

The CBMs performed consistently well on the internal test dataset. The novel β-lactam panel, complete- and minimal panel achieved the highest Youden index (each 0.96), with sensitivities of 100% (95% CI, 100–100%) and specificities of 96% (95% CI, 86–100%) (Table 1). No CPPA was missed, and only 1–2 isolates were flagged for confirmatory testing. These isolates were nCPPA that nevertheless exhibited resistance to ceftolozane-tazobactam. Based on the models' key features (Table 3), these isolates were classified as potential carbapenemase producers by both the minimal- and the novel β-lactam panel model. Since ceftolozane-tazobactam is not included in the standard panel, these isolates do not appear there. Only two nCPPA isolates resistant to ciprofloxacin were misclassified in that instance, as ciprofloxacin is a critical feature in that specific model.

Table 1
Evaluation of the CatBoost-Model with different antibiotic panels and the Gill *et al.* algorithm on the internal test dataset.

	Internal test dataset				
	standard panel	**novel β-lactam panel**	**complete panel**	**minimal panel**	**Gill *et al.***
Sensitivity % (95% CI)	100 (100–100)	**100 (100–100)**	**100 (100–100)**	**100 (100–100)**	100 (96–100)
Specificity % (95% CI)	91 (78–100)	**96 (86–100)**	**96 (86–100)**	**96 (86–100)**	45 (37–53)
Youden index	0.91	**0.96**	**0.96**	**0.96**	0.45
AUROC (95% CI)	0.99 (95–100)	0.98 (93–100)	0.99 (96–100)	0.99 (98–100)	
CPPA total (n =)	14	14	14	14	95
nCPPA total (n =)	23	23	23	23	151
True positive	14 (100%)	14 (100%)	14 (100%)	14 (100%)	94 (98.9%)
True negative	21 (91.3%)	22 (95.7%)	22 (95.7%)	22 (95.7%)	67 (44.4%)
False positive	2 (8.7%)	1 (4.3%)	1 (4.3%)	1 (4.3%)	82 (54.3%)
False negative	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

For the CBM the internal dataset (n = 246) was randomly split into training (70%), validation (15%), and test (15%) sets using stratified sampling. Performance shown for the internal dataset refers exclusively to the held-out test set (n = 37). Algorithms according to Gill et. al was evaluated on the whole internal Dataset (n = 246). Performance metrics are presented as sensitivity, specificity, and Youden index and AUROC with corresponding 95% confidence intervals where applicable. Best results are highlighted in bold.

Table 3
Overview of the most important features of the various antibiotic panels in relation to their discriminatory ability.

complete panel	**standard panel**	**novel β-lactam panel**	**minimal panel**
IMR	CIP	IMR	IMR
CET	ATZ	CET	CET
MEM	AK	AZA	ATZ
TAZ	TAZ	CZA	IMP
AZA	MEM	FPE	MEM

Importance in descending order from top to bottom. IMP, imipenem; IMR, imipenem-relebactam; CIP, ciprofloxacin; CET, ceftolozane-tazobactam; ATZ, aztreonam; CZA, ceftazidime/avibactam; AZA, aztreonam-avibactam; AK, amikacin; MEV, meropenem-vaborbactam; TAZ, ceftazidime; FEP, cefepime; FPE, cefepime-enmetazobactam.

With AUROC values of 0.98–0.99, the CBMs demonstrated excellent capability in distinguishing between CPPA and nCPPA.

The method described by Gill *et al.* achieved a high sensitivity of 100% (95% CI, 96–100%) but lower specificity of 45% (95% CI, 37–53%) with a Youden index of 0.45.

Performance on dataset 1

When the method by Gill *et al.* was assessed, sensitivity and specificity decreased to 94% (95% CI, 74–99%) and 38% (95% CI, 26–51%). This resulted in 52 isolates (70%) being flagged for further testing to identify 17 of 18 CPPAs, with one carbapenemase-producer being missed. For the CBMs, the novel β-lactam panel, complete- and minimal panels showed the best performance, with identical sensitivity (94%), specificity (98%) and Youden indices (0.92), while the corresponding 95% CIs differed slightly.

Thus, only 18 isolates (24.3%) would require further testing to identify 17 CPPA isolates, while one CPPA isolate would be missed. Even with the standard panel, 18 isolates required confirmatory testing, with three CPPA being missed. That means, that a reduction in carbapenemase confirmatory testing of 75.7% can be achieved (Table 2).

Table 2
Evaluation of the CatBoost-Model with different antibiotic panels and method by Gill *et al.* in dataset 1 and 2.

	Dataset 1					**Dataset 2**				
	standard panel	**novel β-lactam panel**	**complete panel**	**minimal panel**	**Gill et al.**	**standard panel**	**novel β-lactam panel**	**complete panel**	**minimal panel**	**Gill et al.**
Sensitivity %	83	**94**	**94**	**94**	94	94	92	**93**	**95**	100
(95% CI)	(61–100)	**(82–100)**	**(81–100)**	**(82–100)**	(74–99)	(88–99)	(85–98)	**(89–97)**	**(90–100)**	(95–100)
Specificity %	95	**98**	**98**	**98**	38	80	98	**100**	**98**	0
(95% CI)	(89–100)	**(95–100)**	**(93–100)**	**(94–100)**	(26–51)	(70–90)	(95–100)	**(100–100)**	**(95–100)**	(0–6)
Youden index	0.78	**0.92**	**0.92**	**0.92**	0.32	0.74	0.90	**0.93**	**0.93**	0
Isolates requiring	18	18	18	18	52	80	65	65	66	95
complementary tests	(24.3%)	(24.3%)	(24.3%)	(24.3%)	(70.3%)	(58.8%)	(47.8%)	(47.8%)	(48.5%)	(69.9%)
Negative isolates requiring	3	1	1	1	35	13	1	0	1	65
complementary tests	(5.4%)	(1.8%)	(1.8%)	(1.8%)	(62.5%)	(20%)	(1.5%)	(0%)	(1.5%)	(100%)
Reduction of confirmatory testing	75.7%	75.7%	75.7%	75.7%	29.7%	40.7%	51.9%	51.9%	49.3%	0%
Total CPPA (n=)	18	18	18	18	18	71	71	71	71	71
Total nCPPA (n =)	56	56	56	56	56	65	65	65	65	65
True positive	15	17	17	17	17	67	64	65	68	71
	(83.3%)	(94.4%)	(94.4%)	(94.4%)	(94.4%)	(95.7%)	(91.4%)	(92.9%)	(95.8%)	(100%)
True negative	53	55	55	55	21	52	64	65	64	0
	(94.6%)	(98.2%)	(98.2%)	(98.2%)	(37.5%)	(80%)	(98.5%)	(100%)	(98.5%)	(0%)
False positive	3	1	1	1	35	13	1	0	1	65
	(5.4%)	(1.8%)	(1.8%)	(1.8%)	(62.5%)	(20%)	(1.5%)	(0%)	(1.5%)	(100%)
False negative	3	1	1	1	1	3	6	5	3	0
	(16.7%)	(5.6%)	(5.6%)	(5.6%)	(5.6%)	(4.3%)	(8.6%)	(7.1%)	(4.2%)	(0%)

Dataset 2 missed values for piperacillin and aztreonam-avibactam. Performance metrics are presented as sensitivity, specificity, and Youden index and AUROC with corresponding 95% confidence intervals where applicable. Best results are highlighted in bold.

Performance on dataset 2

Dataset 2 was more challenging due to a divergent distribution of carbapenemases and the presence of enzymes not included in the internal training dataset (Table S4). The isolates of this collection were resistant to carbapenems and cephalosporins (e.g. ceftazidime and cefepime) with only few exceptions (Figure S3). The main complicating factor was the absence of inhibition zone diameter values for piperacillin and aztreonam-avibactam (AZA). AZA plays an important role for complete and novel β-lactam panel (Table 3). Therefore, the results from dataset 2 can additionally demonstrate how the CBM handles missing values while still achieving robust results. Despite these limitations, the CBMs performed well, with novel β-lactam-, complete- and minimal panel achieving a Youden index of 0.93, with the minimal panel demonstrating the highest sensitivity (95%, 95% CI 90–100%) and the complete panel achieving the highest specificity (100%, 95% CI 100–100%) (Table 2). Of 136 isolates, the complete panel approach flagged 65 isolates (48%) for further testing; this would have resulted in five carbapenemase producers being missed (2x IMP-1, 1x KPC-2, 1x GES-5, 1x NDM-1). The standard panel subjected a total of 80 isolates (59%) to confirmatory testing; however, only three CPPAs would have been missed in this scenario (1x IMP-7, 1x KPC-2, 1x VIM-1). The minimal panel would have resulted in 66 isolates (49%) requiring confirmatory testing and would have missed three CPPA isolates (NDM-1, KPC-2, and GES-5).

In this cohort, the method described by Gill *et al.* was unable to distinguish between CPPA and nCPPA (specificity = 0%) due to resistance to imipenem, ceftazidime, and cefepime (with one exception), while it achieved a sensitivity of 100%.

Most valuable features of the CBMs

The feature importance analysis with the complete panel model demonstrated that the following antibiotics were relevant for differentiation (in descending order): imipenem-relebactam, ceftolozane-tazobactam, meropenem, ceftazidime, and aztreonam-avibactam. The antibiotics included in the standard panel were relevant as follows: ciprofloxacin, aztreonam, amikacin, ceftazidime, and meropenem. The novel β-lactam panel was able to distinguish between CPPA

and nCPPA using imipenem-relebactam, ceftolozane-tazobactam, aztreonam-avibactam, ceftazidime-avibactam, and cefepime-enmetazobactam. The minimal-panel utilized imipenem-relebactam, ceftolozane-tazobactam, aztreonam, meropenem and imipenem for this purpose (Fig. 4, Table 3).

Discussion

The aim of this study was to evaluate whether a CatBoost model based on inhibition zone diameters can reliably predict carbapenemase production in PA and reduce the number of isolates requiring further testing for carbapenemases.

As anticipated, the complete panel CBM, which incorporated the largest number of antibiotics, showed high predictive performance. However, an approach was needed to minimize the number of antibiotics required for prediction. The “novel β-lactam panel” demonstrated that comparable performance could be achieved using seven antibiotics only, highlighting the importance of newer β-lactam/β-lactamase inhibitor combinations for distinguishing carbapenemase production from other resistance mechanisms.

This observation is consistent with the known pharmacological properties of these agents. Ceftolozane retains activity against AmpC-β-lactamases, OprD-loss, and efflux-mediated resistance, but is ineffective against MBL (23). Relebactam inhibits class A- and C β-lactamases and is less affected by OprD-loss and efflux, though it also fails to inhibit MBLs (24). In contrast, aztreonam is stable against MBLs. Furthermore, avibactam inhibits many β-lactamases including AmpC, thereby preserving the activity of AZA in MBL producing isolates (25). Consequently, these agents play a crucial role in carbapenemase detection when a metallo-β-lactamase is present. Based on these findings and with the aim of further reducing the number of antibiotics required for prediction, we selected six agents to constitute a minimal antibiotic panel: cefepime, ceftolozane–tazobactam, imipenem, meropenem, aztreonam, and imipenem–relebactam. Notably, despite this substantial reduction in the number of antibiotics, the minimal panel achieved a performance comparable to that of the larger panels. Nevertheless, even with standard antibiotics, the CBM demonstrated a strong performance, outperforming previously described algorithms.

The most informative features did not differ across panels. The top-ranking features are consistent between the complete, novel β-lactam and minimal-panel configurations, with imipenem-relebactam as the most discriminative feature, followed by ceftolozane-tazobactam (Table 3). While these BL-BLI combinations are absent from the standard panel, ciprofloxacin, aztreonam and amikacin were important discriminators. Interestingly ciprofloxacin and amikacin are not related to the production of carbapenemases. However, considering all isolates from this study, 44% of nCPPA were susceptible to ciprofloxacin and 83% to amikacin, whereas only 2% of CPPA remained susceptible to ciprofloxacin and 26% to amikacin. Similar observations have been previously reported (26, 27). An explanation for this includes the co-localization of carbapenemase genes (bla_{VIM-2}) and aminoglycoside resistance genes (*aacA7, aacC1, aacA4, aacA29a/b*) in Class-1-integrons. This can lead to simultaneous selection driven by selective pressure for carbapenems and aminoglycosides (28). Likewise, the dissemination of high-risk lineages (ST235) could be a reason for an accumulation of resistance against carbapenems, fluoroquinolones, and amikacin (27), although ST235 was detected only 29 times in our study cohort.

In the absence of established guidelines for carbapenemase testing in *P. aeruginosa*, we utilized the algorithm described by Gill *et al.* to establish a baseline for comparison. This algorithm is based on resistance to imipenem or meropenem, combined with additional resistance to ceftazidime and cefepime, or, as a second criterion resistance to imipenem or meropenem and resistance to ceftolozane-tazobactam (Fig. 1). It should be noted that our cohorts were enriched with CPPA, and a majority of isolates exhibited resistance to carbapenems and ceftazidime, also among nCPPA. While the internal training cohort featured a more heterogeneous resistance profile among nCPPA (only 38% exhibiting ceftazidime resistance), both external validation cohorts were enriched with high-level multidrug-resistant isolates in the nCPPA group (85–100% resistance to carbapenems and ceftazidime) (Figure S1-3). Consequently, this overlap in resistance patterns between CPPA and challenging nCPPA cases resulted in low performance of the Gill *et al.* method, yet underscores the robustness of our machine-learning model beyond simple phenotypic thresholds.

Limitations

Several limitations of this study should be acknowledged. First, the isolate collection was restricted to Germany and Switzerland and may therefore not fully reflect the global molecular epidemiology of carbapenemase-producing *P. aeruginosa*. As the CBM is based nearly exclusively on MBL-carbapenemases, the performance for other carbapenemase types remains to be established. However, MBLs, particularly VIM-type enzymes are the most common carbapenemases in PA worldwide (29).

As noted above, the analyzed isolates belonged to cohorts artificially enriched with CPPA, in which even nCPPA were predominantly resistant to carbapenems and cephalosporins. Consequently, the results represent an extreme scenario, and improved model performance in routine clinical settings is likely. Prospective validation in a real-world setting is necessary.

The strength of this study is the large collection of molecularly characterized isolates used for the training of the model and the validation on two external datasets, demonstrating that the CBM performs well even when on data from other regions and on incomplete data.

Disk diffusion was chosen as the susceptibility testing method because it has a wide range of inhibition zone data, is commonly used and recommended by CLSI and EUCAST. Semi-automated methods are also frequently used in the routine setting, but often have a limited calling range of MICs and their layouts differ between countries, complicating the development of a model which is universally applicable.

Conclusion

Compared to standard algorithms, CatBoost models represent a valuable tool for substantially reducing the number of confirmatory tests required when carbapenemase production is suspected in *P. aeruginosa*. To the best of our knowledge, it is the first ML-based open-access web-app for the prediction of

carbapenemase production in *P. aeruginosa*. Even with standard antibiotics used in the therapy of PA-Infections, the CBM demonstrated an excellent performance and is easy to use. The integration of the CBM into the CarbaDetector web-tool is straightforward and delivers rapid results, offering significant potential for cost and time savings in routine diagnostics, especially in resource-limited settings.

Abbreviations

PIP – piperacillin

TZP – piperacillin-tazobactam

TAZ – ceftazidime

CBM – CatBoost Model

CZA – ceftazidime-avibactam

CET – ceftolozane-tazobactam

FEP – cefepime

CID – cefiderocol

FPE – cefepime-enmetazobactam

ATZ – aztreonam

AZA – aztreonam-avibactam

IMP – imipenem

IMR – imipenem-relebactam

MEM – meropenem

MEV – meropenem-vaborbactam

CIP – ciprofloxacin

AK – amikacin

BL-BLI – β-lactam-β-lactamase inhibitor

Declarations

Competing Interests

Axel Hamprecht has received research support from bioMérieux and Bruker and served on the advisory board of bioMérieux.

Lukas Schaffarczyk has received research support (consumables) from Liofilchem.

All other authors have no relevant financial or non-financial interests to disclose.

Ethics approval

The bacterial strains were obtained during routine diagnostics and anonymized prior to analysis. No patient-related data were collected or analyzed. According to §15 of the Professional Code for Physicians of the Medical Association of Lower Saxony, consultation by an ethics committee was therefore not required. Strains from dataset 2 could be used for development and testing of new diagnostic tools and do not fall under the Swiss Human Research Act, as no patient related data was used and all samples were anonymized.

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Author Contribution

Lukas Schaffarczyk: Conceptualization, methodology, formal analysis; writing – original draft; writing – review & editing.

Linea Muhsal: methodology, writing – review & editing.

Rebekka Laufenberg: resources, review & editing.

Niels Pfennigwerth: resources, review & editing.

Sören Gatermann: resources, review & editing.

Stefano Mancini: resources, review & editing.

Adrian Egli: resources, review & editing.

Oliver Nolte: resources, review & editing

Nils Strodthoff: methodology, formal analysis, writing – review & editing.

Axel Hamprecht: Conceptualization, resources, methodology, review & editing.

Data availability statement

All data supporting the findings of this study are available within the manuscript and its Supplementary Information. Raw sequencing data have been deposited in the Sequence Read Archive (SRA) of the National Centre for Biotechnology Information under BioProject accession number PRJNA1459623. Individual accession numbers are provided in Tables S2 and S3. Model code is available as a github repositorie.

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Figures

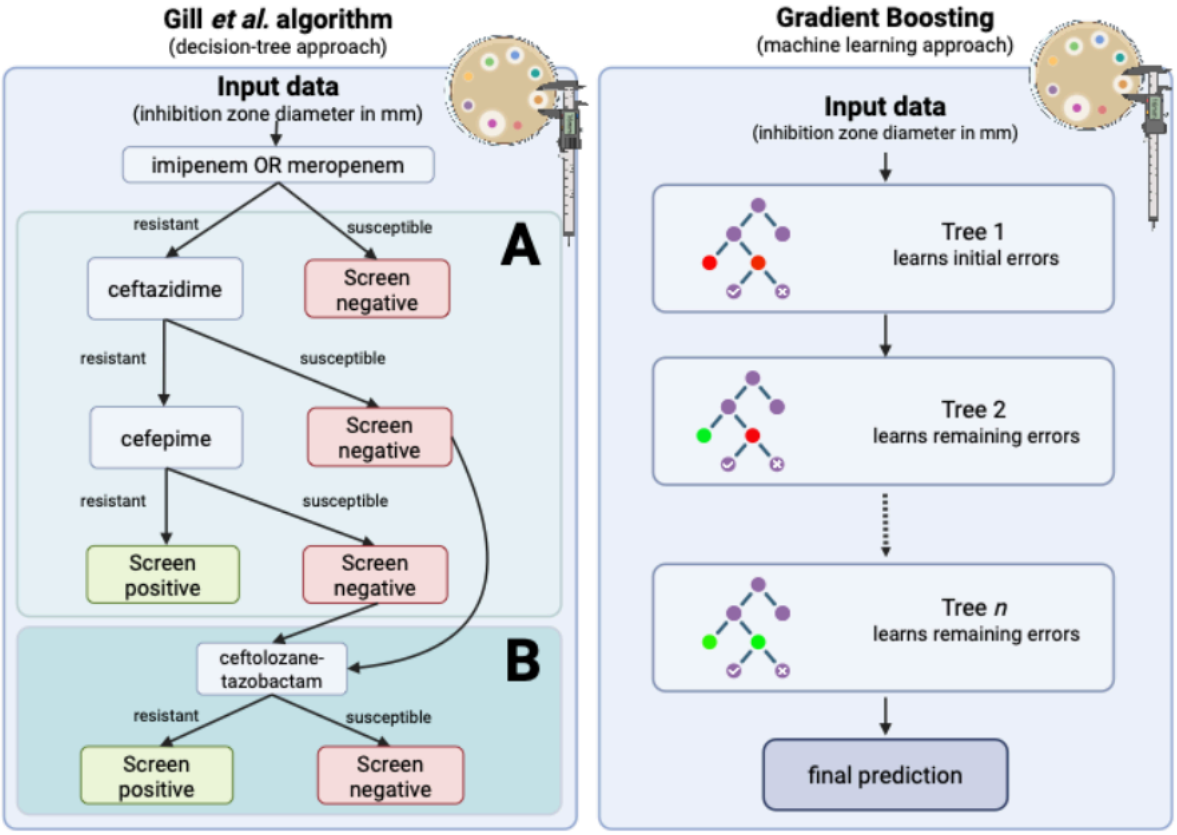


Figure 1

Comparison of the algorithm of Gill et al. with the principle of the machine learning approach ”gradient boosting“

„A“ represents the primary algorithm criterion by Gill *et al*., while „B“ represents the secondary ceftolozane-tazobactam-based criterion.

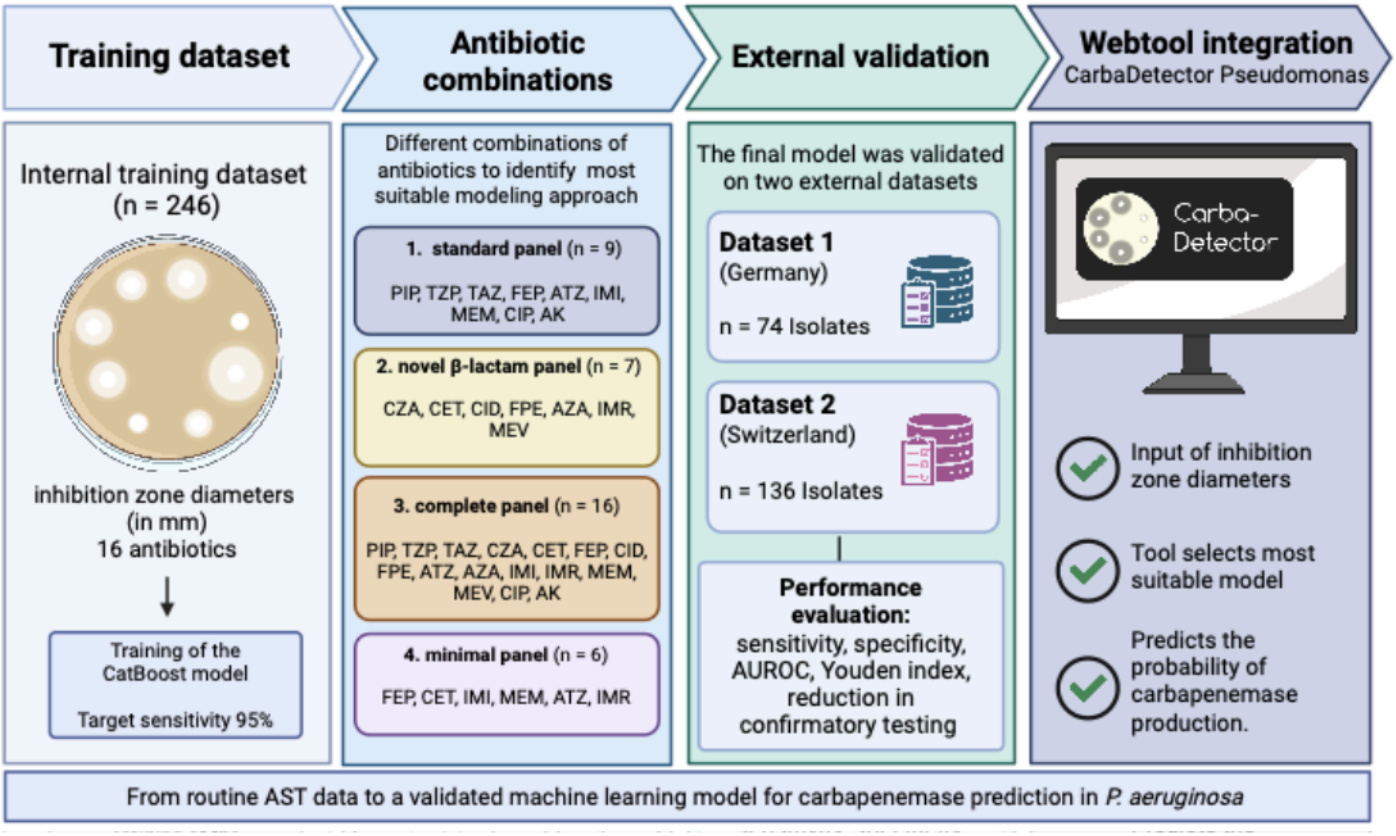


Figure 2

Development, validation and web tool integration of the CatBoost model „CarbaDetector Pseudomonas“



Figure 3

Interface of the „CarbaDetector Pseudomonas“ web-app.

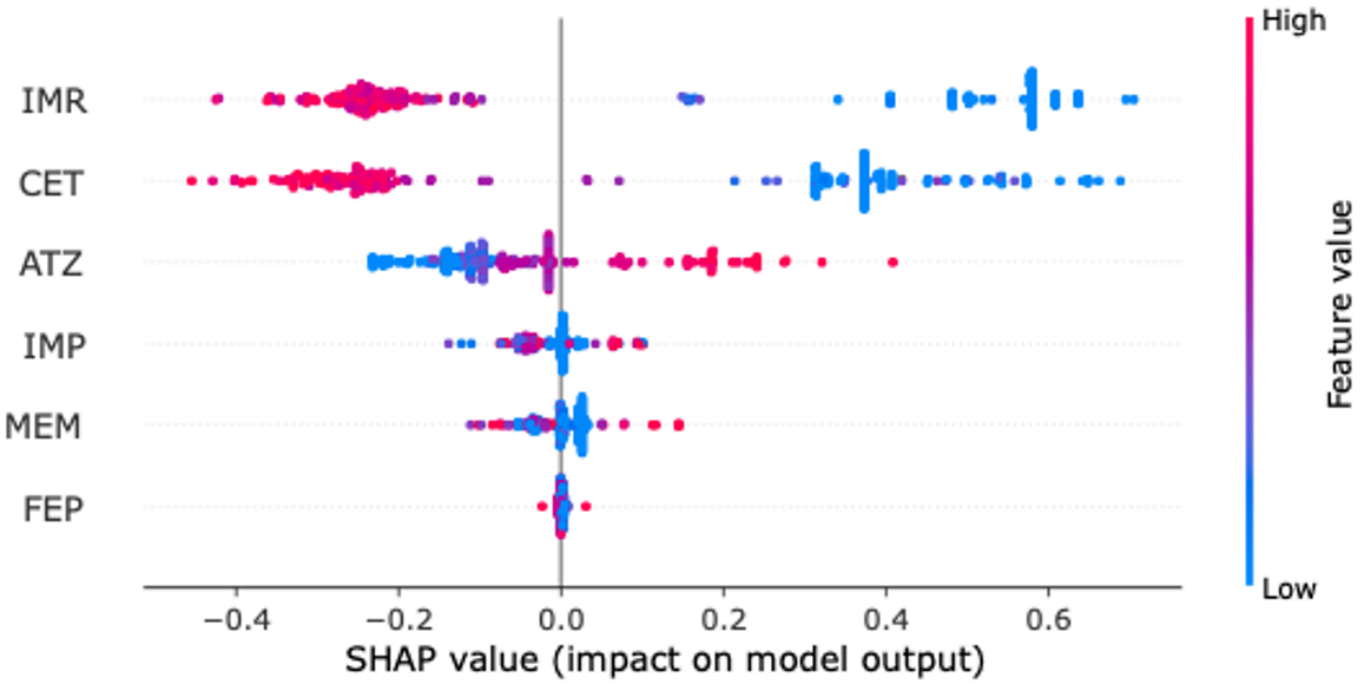


Figure 4

SHAP feature importance for the minimalpanel model evaluated on the internal training set

Indicates which features were crucial to distinguish between CPPA and nCPPA. IMR, imipenem-relebactam; CET, ceftolozane-tazobactam; AZA, aztreonam-avibactam; MEV, meropenem-vaborbactam; AK, amikacin; CIP, ciprofloxacin; CZA, ceftazidime/avibactam; ATZ, aztreonam; TAZ, ceftazidime

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