

1 **Rapidly predicting vancomycin resistance of *Enterococcus faecium* through**
2 **MALDI-TOF MS spectrum obtained in real-world clinical microbiology**
3 **laboratory**

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40 **Keywords:** Vancomycin-resistant *Enterococcus faecium* (VRE_{fm}); Antibacterial drug
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42 spectrometry; Machine learning; Rapid detection

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50 **Abstract**

51 *Enterococcus faecium* is one of the leading pathogens in the world. In this study, we proposed
52 a strategy to rapidly and accurately distinguish vancomycin-resistant *Enterococcus faecium*
53 (VRE_{fm}) and vancomycin-susceptible *E. faecium* (VSE_{fm}) to help doctors correctly determine
54 the use of vancomycin by a machine learning (ML)-based algorithm. A predictive model was
55 developed and validated to distinguish VRE_{fm} and VSE_{fm} by analyzing MALDI-TOF MS
56 spectra of unique *E. faecium* isolates from different specimen types. Firstly, 5717 mass spectra,
57 including 2795 VRE_{fm} and 2922 VSE_{fm}, were used to develop the algorithm. And 2280 mass
58 spectra of isolates, namely 1222 VRE_{fm} and 1058 VSE_{fm}, were used to externally validate the
59 algorithm. The random forest-based algorithm demonstrated good classification performances
60 for overall specimens, whose mean AUROC in 5-fold cross validation, time-wise validation,
61 and external validation was all greater than 0.84. For the detection of VRE_{fm} in blood, sterile
62 body fluid, urinary tract, and wound, the AUROC in external validation was also greater than
63 0.84. The predictions with algorithms were significantly more accurate than empirical
64 antibiotic use. The accuracy of antibiotics administration could be improved by 30%. And the
65 algorithm could provide rapid antibiotic susceptibility results at least 24 hours ahead of routine
66 laboratory tests. The turn-around-time of antibiotic susceptibility could be reduced by 50%. In
67 conclusion, a ML algorithm using MALDI-TOF MS spectra obtained in routine workflow
68 accurately differentiated VRE_{fm} from VSE_{fm}, especially in blood and sterile body fluid, which
69 can be applied to facilitate the clinical testing process due to its accuracy, generalizability, and
70 rapidness.

71

72 **Introduction**

73 *Enterococcus* spp. is one of the leading pathogens in healthcare-associated infection.¹
74 Enterococcal infection could cause urinary tract infection, blood stream infection, and even
75 mortality.² Until recently, vancomycin was virtually the only drug that could be consistently
76 relied on for treating multidrug-resistant enterococcal infections^{3,4}. Vancomycin-resistant
77 *Enterococcus* (VRE) has led to heavy burden on healthcare worldwide since its first-time
78 isolation.^{5,6} *Enterococcus faecalis* and *E. faecium* are the 2 most commonly isolated
79 *Enterococcus* spp. in clinical practice.¹ VRE *faecium* (VRE_{fm}) has received considerably more
80 attention than VRE *faecalis* (VRE_{fs}) because most of the clinically isolated VRE is *E. faecium*
81 in the recent decades^{4,7} and VRE_{fm} causes more severe infection than VRE_{fs}^{8,9}. Early detection
82 of vancomycin resistance is essential for successfully treating VRE_{fm} infection.¹⁰ Vancomycin
83 could be discontinued, and antimicrobial agents could be replaced with other antibiotics (eg,
84 linezolid and daptomycin) based on the laboratory results of vancomycin resistance^{11,12}.
85 Patients' prognosis could be improved and further drug resistance development could be
86 avoided by using susceptible antibiotics.¹¹ However, typical tests in clinical microbiology
87 laboratories, such as the minimal inhibitory concentration test or agar-diffusion test, fail to
88 provide results for antibiotic susceptibility rapidly. The antibiotic susceptibility test (AST) of
89 vancomycin is time-consuming, and the Clinical and Laboratory Standards Institute
90 recommended a full 24 hours should be held for accurate detection of vancomycin resistance
91 in enterococci.¹³ This would considerably delay accurate prescription of antibiotics against *E.*
92 *faecium*. Furthermore, prescribing antibiotics based on empirical prescription, without
93 determining AST, would result in low effectiveness (approximately 50%), depending on the
94 local epidemiology of VRE_{fm}.¹² Thus, a new tool is needed to provide AST for VRE_{fm} rapidly
95 and accurately.

96 Recently, matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass
97 spectrometry (MS) has become popular among clinical microbiology laboratories worldwide
98 because of its reliability, rapidity, and cost-effectiveness in identifying bacterial species.¹⁴⁻¹⁶ In
99 addition to species identification, MALDI-TOF MS has been promising in other applications,
100 such as strain typing or AST.¹⁷⁻¹⁹ MALDI-TOF MS can generate massive data comprising
101 hundreds of peak signals on the spectra.^{17,20} The complex data of MALDI-TOF spectra are
102 overwhelming to even an experienced medical staff.¹⁹ Studies have attempted to identify the
103 characteristic peak through visual inspection.^{21,22} The results of the studies have been
104 discordant, which has limited the clinical utility.²³⁻²⁵

105 Machine learning (ML) is a good analytical method for solving classification problems
106 through identification of implicit data patterns from complex data.²⁶ The ML method
107 outperforms traditional statistical methods because of its excellent ability to handle complex
108 interactions between large amount of predictors and good performance in non-linear
109 classification problems²⁷ ML has been successfully applied in several clinical fields.²⁷⁻³⁶ Thus,
110 the ML algorithm is especially appropriate for analyzing complex data such as MALDI-TOF
111 spectra. However, to our knowledge, few studies have used ML in the analysis of MALDI-
112 TOF spectra for rapidly reporting *VREfm*, and the case numbers in these studies were
113 insufficient, and so, ML algorithm generalization has been limited.³⁷⁻³⁹ Moreover, to date, no
114 study has validated AST prediction ML algorithms by using large real-world data.

115 In this study, we aimed to develop and validate a *VREfm* prediction ML model by using
116 consecutively collected real-world data from 2 tertiary medical centers (Chang Gung Memorial
117 Hospital [CGMH], Linkou branch and CGMH, Kaohsiung branch). Using the largest MALDI-
118 TOF spectrum clinical data to date, the ML algorithm could predict *VREfm* accurately, rapidly,
119 and in a ready-to-use manner based on the real-world evidence, which is more representative
120 for clinical practice.⁴⁰ Moreover, we confirmed the robustness and generalization of the ML

121 algorithm through several validation methods, namely cross-validation, time-wise internal
122 validations (unseen independent testing dataset classified according to time), and external
123 validation (unseen independent testing dataset from another medical center). According to the
124 real-world evidence-based validation, our VRE fm prediction ML models are ready to be
125 incorporated into routine workflow.

126 **Materials and methods**

127 **Data source**

128 We designed a novel machine learning approach which can improve accuracy of
129 antibiotics administration and reduce the turn-around-time of antibiotics susceptibility test. We
130 summarized the comparison between the machine learning approach and the traditional
131 approach used in current clinical microbiology laboratory. We schematically illustrated the
132 study design in Figure 1(b). The data used in this retrospective study was consecutively
133 collected from the clinical microbiology laboratories of 2 tertiary medical centers in Taiwan,
134 namely CGMH Linkou branch and CGMH Kaohsiung branch between January 1, 2013 and
135 December 31, 2017. The clinical microbiology laboratories collected and processed all the
136 routine specimens obtained from the hospitals. In total, 7997 *E. faecium* cases were identified
137 and included in this study, whereas 5717 (VRE fm : 48.89%) and 2280 (VRE fm : 53.60%) cases,
138 respectively, were obtained from Linkou and Kaohsiung branches of CGMH. The *E. faecium*
139 strains were isolated from blood, urinary tract, sterile body fluids, and wound. The detailed
140 description of specimen types is provided in eTable 1 in the Supplement. The study was
141 approved by the Institutional Review Board of Chang Gung Medical Foundation (No.
142 201900767B0). We followed the Standards for Reporting of Diagnostic Accuracy 2015⁴¹ and
143 the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or
144 Diagnosis reporting guidelines.⁴²

145

146 **Definition of *E. faecium* and vancomycin susceptibility**

147 *E. faecium* was identified using MALDI-TOF spectra measured using a Microflex LT
148 mass spectrometer and analyzed using Biotyper 3.1 (Bruker Daltonik GmbH, Bremen,
149 Germany). A log score (generated through Biotyper 3.1) larger than 2 was used for confirming
150 *E. faecium*.¹⁷⁻¹⁹ We tested vancomycin susceptibility of *E. faecium* by using the paper disc
151 method. The details of *E. faecium* identification and AST are given in the eMethods in the
152 Supplement.

153

154 **MALDI-TOF mass spectrum data collection and preprocessing**

155 The details were described in the Supplements.

156

157 **Peak selection from MALDI-TOF mass spectra for model development**

158 We applied the embedded feature-selection method to select the most important peaks
159 from MALDI-TOF mass spectra.⁴³ The peaks were ranked using the p-values of the chi-square
160 test of homogeneity, which was employed to determine whether frequency counts were
161 distributed identically across VRE_{fm} and vancomycin-susceptible *E. faecium* (VSE_{fm}).
162 Preliminarily, we selected top 10 important peaks to plot a heat map based on the hierarchical
163 clustering (eMethods in the Supplement). All the ranked peaks were incorporated in the model
164 accordingly until the performance did not increase. Consequently, we could obtain the
165 important peaks that were highly related to differentiation of VRE_{fm} and VSE_{fm} isolates.

166 For determining the number of peaks included in the ML models, we forwardly added
167 them into the ML models and calculated the performance using accuracy as the metric. First,
168 the predictor candidates were sorted in a descending order according to the importance score,
169 and one predictive peak was added at a time into the ML models. On the basis of predictive
170 peak composition, we used different algorithms, namely random forest (RF), support vector

171 machine (SVM) with a radial basis function kernel, and k-nearest neighbor (KNN) and applied
172 5-fold cross validation (CV) in the data from the CGMH Linkou branch. The accuracies of the
173 ML models were calculated to determine the adequate number of predictive peaks included in
174 the models.

175

176 **Development and validation of VRE_{fm} prediction models**

177 We aimed to develop and validate a robust VRE_{fm} prediction model capable of
178 detecting VRE_{fm} earlier than the AST report. Three commonly used ML algorithms, namely
179 RF, SVM with a radial basis function kernel, and KNN, were used for developing the VRE_{fm}
180 prediction model. These ML algorithms have demonstrated their successful applications (either
181 classification or prediction) in clinical practice.^{17-19,27,28,35,36} The details of these ML algorithms
182 and model training processes are attached in the eMethods in the Supplement.

183 We thoroughly evaluated the performance and robustness of the VRE_{fm} prediction
184 models using 5-fold CV, time-wise internal validation, and external validation. Data from the
185 CGMH Linkou branch were used for 5-fold CV and time-wise internal validation; by contrast,
186 data from the CGMH Kaohsiung branch served as the unseen independent testing data for
187 external validation. For 5-fold CV, data were randomly divided into 5 datasets. Each one of the
188 5 datasets served as the testing dataset to evaluate the performance of the model developed by
189 the other 4 datasets. In 5-fold CV, we obtained 5 measurements of metrics for evaluating the
190 robustness of VRE_{fm} prediction models. Moreover, to evaluate performance using
191 prospectively collected data, we conducted time-wise internal validation: we used data
192 collected between January 1, 2013 and December 31, 2016 as the training dataset for
193 developing VRE_{fm} prediction models, while data from January 1, 2017 to December 31, 2017
194 served as the testing dataset. To test the generalizability of the models, we used data from the
195 CGMH Linkou branch to develop VRE_{fm} prediction models and used data from the CGMH

196 Kaohsiung branch to test the models' performance in a different institute. Additionally, we
197 evaluated the performance of the *VRE_{fm}* prediction model using different types of specimens,
198 namely blood, urinary tract, sterile body fluid, and wound, by using data from the CGMH
199 Kaohsiung branch. We adopted metrics including sensitivity, specificity, accuracy, positive
200 predictive value (PPV), negative predictive value (NPV), receiver operating characteristic
201 (ROC) curve, and area under the receiver operating characteristic curve (AUROC) to assess
202 and compare the performance of the *VRE_{fm}* prediction model.

203

204 **Statistical analysis**

205 The confidence intervals for sensitivity, specificity, and accuracy were estimated
206 using the calculation of the confidence interval for a proportion in one sample situation.
207 Specifically, the critical values followed the Z-score table. To compare the percentages in
208 matched samples, Cochran's Q test, a nonparametric approach, was implemented in this
209 study.⁴⁴ Then, we employed pairwise McNemar's tests⁴⁵ for post hoc analysis and adopted
210 the false discovery rate proposed by Benjamini and Hochberg (1995) to adjust the *P* value.⁴⁶
211 Furthermore, the confidence intervals of AUROCs were determined using the nonparametric
212 approach, and the AUROC comparisons mainly adopted the nonparametric approach
213 proposed by DeLong et al.⁴⁷

214

215 **Results**

216 **Predictive peaks for detecting *VRE_{fm}***

217 We defined crucial predictive peaks when the occurrence frequency of a peak was
218 significantly different (defined by the chi-square test) in *VRE_{fm}* and *VSE_{fm}*. In the step of
219 extracting predictor candidates, 876 predictor candidates were extracted. From the predictor
220 candidates, we used the chi-square method to select important predictive peaks.

221 We selected 10 most critical predictive peaks and plotted a heat map to preliminarily
222 visualize the difference between *VREfm* and *VSEfm* (Figure 2). Peaks of m/z 3172, 3302, 3645,
223 6342, 6356, 6603, and 6690 were found more frequently in *VREfm*; by contrast, m/z 3165,
224 3681, and 7360 occurred more frequently in *VSEfm*. Although these important predictive peaks
225 were statistically significant, we found them in both *VREfm* and *VSEfm*. The full list of crucial
226 predictive peaks is provided in eTable 2 in the Supplement.

227 We selected several important predictive peaks from the predictor candidate list, which
228 was ordered according to the chi-square score. eFigure 4 in the Supplement shows the change
229 in ML models performance when the number of critical predictive peaks increased. For all the
230 ML algorithms used in the study, a similar trend of performance was observed: the accuracies
231 of the ML models reached a steady plateau when the included number of important predictive
232 peaks was larger than 100 (eFigure 4 in the Supplement). Thus, the top 100 crucial predictive
233 peaks were selected as the peak composition for the following experiments.

234

235 **Performance of *VREfm* prediction models**

236 We summarized the ML models' performance in Table 1, Table 2, and Figure 3. The
237 details of comparison between different algorithms are described in the Supplement. The RF
238 model outperformed SVM and KNN in 5-fold CV, time-wise internal validation, and external
239 validation (eTable 3 in the Supplement), where the AUROC ranged from 0.8463 to 0.8553 and
240 accuracy ranged from 0.7769 to 0.7855. Moreover, performance robustness was also observed
241 in SVM and KNN. Figure 3 shows typical ROC curves for the 3 algorithms in all the 3
242 validations. We used Youden's index to select the threshold from the ROC curves in search of
243 balanced sensitivity and specificity. In external validation, the sensitivity and specificity of RF
244 were 0.7791 (95% confidence interval: 0.7620-0.7961) and 0.7930 (95% confidence interval:

245 0.7764-0.8096). On the basis of the resistance rate (VRE_{fm}: 53.60%) in the external validation
246 dataset, the PPV was 0.8130 and the NPV was 0.7565.

247 Given that the RF algorithm attained the highest performance, additionally, we tested
248 the performance of the RF-based VRE_{fm} prediction model using different types of specimens
249 in the independent testing dataset (ie, external validation by using data of the CGMH
250 Kaohsiung branch) (Table 2). The RF-based VRE_{fm} prediction model attained higher
251 performance in predicting VRE_{fm} in blood and sterile body fluid specimens than the other
252 specimen types. The AUROC of blood specimens reached 0.9103 (95% confidence interval:
253 0.8727-0.9480), whereas that of sterile body fluid specimens reached 0.8714 (95% confidence
254 interval: 0.8321-0.9106). Moreover, the sensitivity (0.8870, 95% confidence interval: 0.8436-
255 0.9303) and specificity (0.8000, 95% confidence interval: 0.7452-0.8548) of the RF-based
256 VRE_{fm} prediction model for the blood specimen were also balanced and significantly higher
257 than those for other specimens. By contrast, the performance of the RF-based VRE_{fm}
258 prediction model for urinary tract specimens (0.8494, 95% confidence interval: 0.8258-0.8731)
259 was similar to that for overall specimens (0.8553, 95% confidence interval: 0.8399-0.8706).

260

261 **Discussion**

262 We developed ML-based models for predicting VRE_{fm} rapidly and accurately based
263 on MALDI-TOF MS data. The models were especially effective in predicting VRE_{fm} in
264 invasive infections (ie, blood and sterile body fluid). We used the largest up-to-date real-world
265 data to validate the robustness and generalization of the ML-based models by using k-fold CV,
266 time-wise internal validation, and external validation. The rapid and accurate AST of
267 vancomycin is promising for determining antibiotics against VRE_{fm} infection.

268 Our results suggested that AST could be predicted accurately by using ML algorithms
269 to analyze MALDI-TOF MS data. MALDI-TOF MS is a powerful analytical tool in current

270 clinical microbiology laboratories because of its rapidness and cost-effectiveness in identifying
271 bacterial species.¹⁴⁻¹⁶ On the basis of the massive data produced by MALDI-TOF MS,
272 moreover, some studies have demonstrated that subspecies typing could be predicted from a
273 specific pattern of MS spectra only.^{17,19} Furthermore, other studies have shown a good
274 correlation between AST and specific patterns of MS spectra.^{18,23-25,48} However, some issues
275 have limited the generalization of these results. First, most of the studies have adopted an
276 additional protein extraction step before analytical measurement of MALDI-TOF MS. The
277 protein extraction step could enhance data quality; however, it is not routinely used in clinical
278 practice because it is labor-intensive, time-consuming, and expensive.^{17,18} By contrast, we used
279 the direct deposition method, which is recommended by the manufacturer and is used for
280 everyday works. Thus, our models are more feasible for the existing workflow because they
281 were trained using real-world data. Second, the data sizes in these studies were too small to be
282 representative. We demonstrated that the ML-based models for predicting *VRE_{fm}* can be
283 applied as a clinical decision support tool by using the largest up-to-date datasets collected
284 through the direct deposition method and various validation methods.

285 Identifying crucial predictive peaks in *VRE_{fm}* classification may not be essential in
286 clinical application; however, the specific combination of crucial predictive peaks would
287 inspire further studies investigating the molecular mechanism of *VRE_{fm}*. Typically, the *vanA*
288 cluster is the most common mediator of vancomycin resistance in enterococci,⁴⁹ although many
289 vancomycin resistance genes have been identified.⁵⁰ In brief, many factors together attribute to
290 antibiotic resistance. Moreover, the complex mechanisms of antibiotic resistance would evolve
291 in response to the selective pressures of their competitive environment (eg, antibiotic use).⁴⁹
292 Thus, identifying the important predictive peaks for *VRE_{fm}* could help us understand the
293 mechanism behind resistance. In this study, for example, peaks of *m/z* 6603, 6631, and 6635
294 were found frequently for *VRE_{fm}* (eTable 2 in the Supplement). The finding is consistent with

295 a previous study where Griffin et al. reported m/z 6603 is specific for *vanB*-positive *VREfm*,
296 while m/z 6631 and 6635 are specifically found for *vanA*-positive *VREfm*.³⁸ These peaks are
297 worthy of further identification in future investigations. Moreover, new antibiotics against
298 *VREfm* can be developed based on these predictive peaks for *VREfm*.

299 Our ML models persistently performed well in 5-fold CV, time-wise internal validation,
300 and external validation. Moreover, all the ML algorithms used in this study exhibited good
301 performance (AUROC > 0.8). It could be explained that discriminating *VREfm* from *VSEfm*
302 is generally achievable after adequate feature extraction and feature selection processes. In
303 time-wise internal validation, we intended to simulate a prospective study for a model trained
304 by the “past data” to analyze the “future data.” Based on the performance of time-wise internal
305 validation, we concluded that the trained ML models could also perform well on the
306 prospectively collected data, which are unseen in the training process. Previous study results
307 differentiating *VREfm* from *VSEfm* by using MALDI-TOF MS spectra could not be
308 generalized.^{23-25,38} The inconsistent results could be because less features (<10) were used. A
309 review article reported that peak-level reproducibility of MALDI-TOF mass is approximately
310 80%.⁵¹ The classification performance is compromised when essential peaks are few and
311 happen to be absent on the mass spectra. In our study, the ML models performed stably when
312 the included peaks were more than 100 (eFigure 4 in the Supplement). The steady and good
313 performance of our ML models could be explained by the much more included peaks: when
314 some of the essential peaks are not reproduced in the mass spectra, we can still use other
315 alternative essential peaks to conduct an accurate classification. The number of essential peaks
316 somehow compensated the insufficient reproducibility of MALDI-TOF mass. By contrast,
317 regarding predicting *VREfm* for various specimens, we found that the RF-based model
318 performed especially well in blood and sterile body fluids. The superior prediction performance
319 could be attributed to the relatively fewer number of *VREfm* strains in blood and sterile body

320 fluids. Bacterial infection in blood or sterile body fluids is typically regarded as invasive
321 infection.⁵² Only a few *VREfm* strains (sequence type (ST)17, ST18, ST78, and ST203) cause
322 invasive infections in blood or sterile body fluids according to the studies in Taiwan⁵³ and
323 Ireland.⁵⁴ The nature of the classification problem would be more simple when the number of
324 labels is fewer.

325

326 **Limitations**

327 This study has several limitations. First, although the models were evaluated using
328 unseen external data from different medical centers, all the training data and testing data were
329 collected from only 2 tertiary medical centers in Taiwan. Directly applying the ML models in
330 hospitals of other areas or countries as well as in primary care institutes may not be appropriate.
331 However, we believe that the method, but not the trained model, could be generalized.
332 Although our ML models were validated comprehensively using 3 different approaches and
333 the results show that the difference in MALDI-TOF mass spectra between *VREfm* and *VSEfm*
334 can be distinguished through all the ML algorithms we used, we suggest others collecting their
335 locally relevant data for training and validating the *VREfm* predicting model given that the
336 epidemiology of *VREfm* could be fairly different site by site. Second, our primary goal was to
337 develop and validate a practical and ready-to-use ML model in real-world practice. We found
338 some crucial predictive peaks for *VREfm*; however, we did not confirm the identities for these
339 peaks. It is worthy of identifying these peaks in further investigations. Third, we did not use
340 the deep learning (DL) algorithm for predicting *VREfm*, although DL has been successful in
341 the image classification or radiology field.^{32,33} In this study, *VREfm* could be accurately
342 predicted using several classic algorithms (ie, RF, SVM, and KNN) that require less resource
343 and time in training and using models. Moreover, DL usually requires more training samples
344 and is financially and computationally more expensive than classical ML algorithms.⁵⁵ DL

345 utility in analyzing MS data rather than image data could be another promising issue in the
346 bioinformatics field. Fourth, no strain typing data were included. Thus, the molecular
347 epidemiology of *VRE_{fm}* used in this study is unknown.

348

349 **Conclusions**

350 We developed and validated robust ML models capable of discriminating *VRE_{fm}* from
351 *VSE_{fm}* based on MALDI-TOF MS spectra. These models were especially good at detecting
352 *VRE_{fm}* causing invasive diseases. The accurate and rapid detection of *VRE_{fm}* by using the ML
353 models would facilitate more appropriate antibiotic prescription.

354

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357

358 **Author Contributions**

359 HYW, KPL, and CRC had full access to all the data in the study and take responsibility for the
360 integrity of the data and the accuracy of data analysis. HYW, KPL, CRC, and YJT
361 analyzed/interpreted the data, performed experiments, designed the study, and wrote the
362 manuscript. HYW, CRC, YJT, JTH, TYL, THC, MHW, TPL, and JLL reviewed/edited the
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364 material support. JLL obtained funding and supervised the study.

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371

372 **Competing interests**

373 The authors have no affiliations with or involvement in any organization or entity with any
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376

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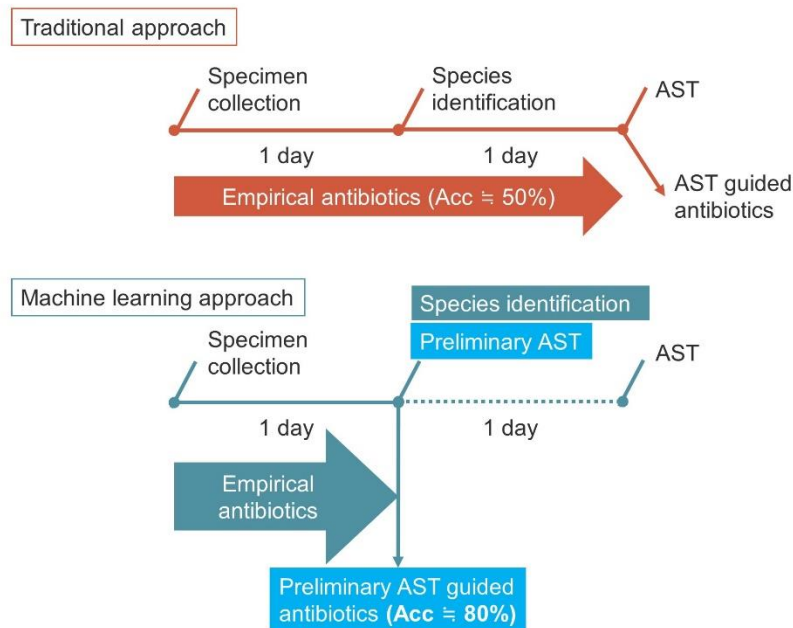
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552

553 Figure legends

Figure 1(a)



554

555 **Figure 1(a). Scheme of using the VRE_{fm} Model.** We plotted a timeline of bacterial culture

556 test in current clinical microbiology laboratory (i.e., traditional approach) and a

557 modified timeline when the VRE_{fm} model is incorporated (i.e., machine learning

558 approach). In the traditional approach, specimens are collected for bacterial culture

559 test. One day is usually needed for growth of a single colony for species

560 identification (by MALDI-TOF MS). Antibiotics susceptibility test (AST) of

561 vancomycin for VRE_{fm} will cost another day to report. By contrast, in the machine

562 learning approach, the VRE_{fm} model can provide preliminary AST at the time when

563 bacterial species is identified by MALDI-TOF MS. For treating VRE_{fm}, the machine

564 learning approach can improve accuracy of antibiotics use by around 30% (from

565 50% accuracy of empirical antibiotics use in the traditional approach to 80%

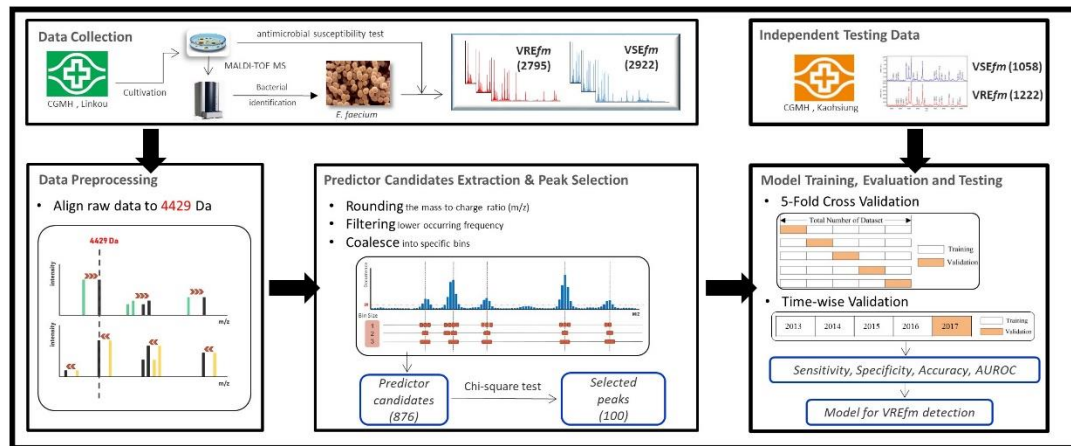
566 accuracy of preliminary AST provided by the machine learning approach).

567 Meanwhile, the turn-around-time of bacterial culture test can be reduced to one day,

568 which is 50% reduction.

569

Figure 1(b)



570

571 **Figure 1(b). Schematic Illustration of the Study Design.** We developed and validated a VRE_{fm}

572 prediction model. The study included several steps, namely data collection, data

573 preprocessing, predictor candidate extraction and important predictor selection, model

574 training, evaluation, and testing. In data collection, data were obtained from 2 tertiary

575 medical centers (Linkou and Kaohsiung branches of CGMH). The data included mass

576 spectra and results of the vancomycin susceptibility test of *E. faecium*. Data from the CGMH

577 Linkou branch were used for model training and validation, while data from the CGMH

578 Kaohsiung branch served as an independent testing data. In the steps of data preprocessing

579 and predictor candidate extraction and important predictor selection, a specific set of crucial

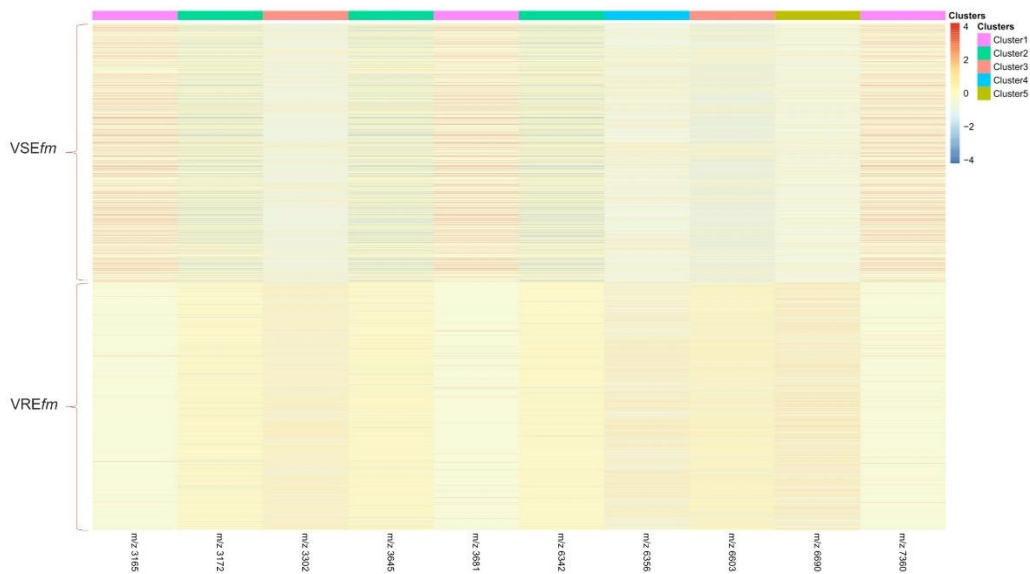
580 predictors would be used for model training. K-fold, time-wise CV, and external validation

581 were used to confirm the models' robustness. The VRE_{fm} prediction model can detect

582 VRE_{fm} accurately at least 1 day earlier than the current method.

583

Figure 2



584

585 **Figure 2. Heat map.** We selected top 10 discriminative peaks by chi-square testing the occurrence

586 frequency of peaks in VREfm and VSEfm. The heat map was plotted based on the hierarchical

587 clustering of all the VREfm and VSEfm isolates from the CGMH Linkou branch. Rows

588 represent the isolates, and columns represent the top 10 discriminative peaks. The values in

589 the heat map represent the MS spectral intensity which was log₁₀-normalized and z-score

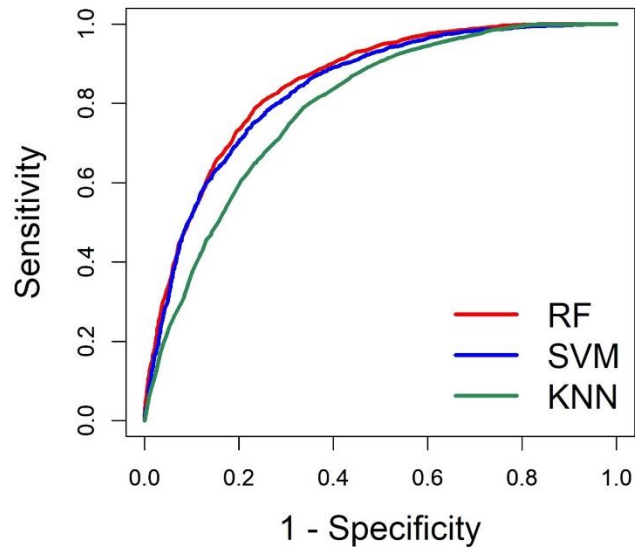
590 standardized. Red color indicates relatively higher peak intensity while blue color indicates

591 lower peak intensity. The isolates are grouped into 5 clusters. VREfm and VSEfm isolates can

592 be visually differentiated by using the top 10 discriminative peaks.

593

Figure 3(a)

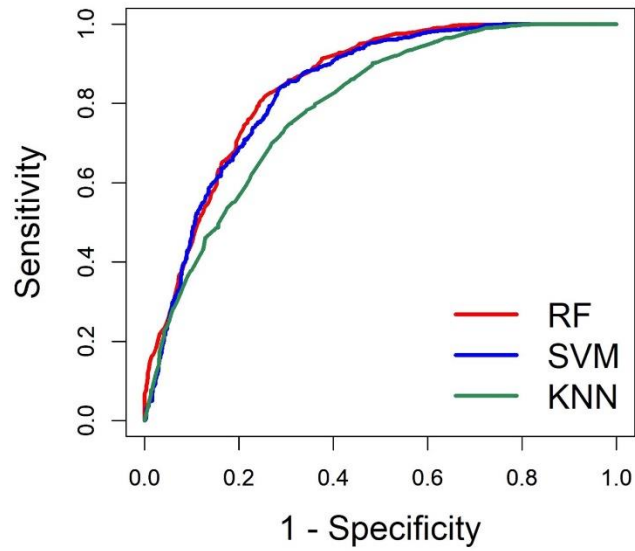


594

595 **Figure 3(a). ROC Curves for Different Algorithms in Terms of Linkou 5-Fold CV**

596

Figure 3(b)

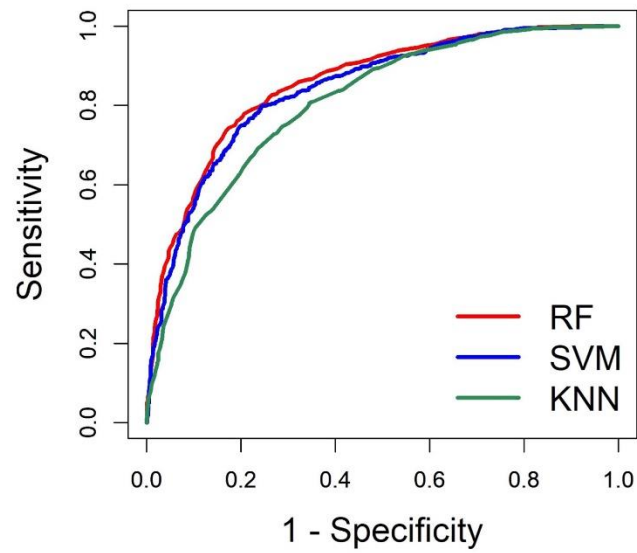


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598 **Figure 3(b). ROC Curves for Different Algorithms in Terms of Time-Wise Validation**

599

Figure 3(c)

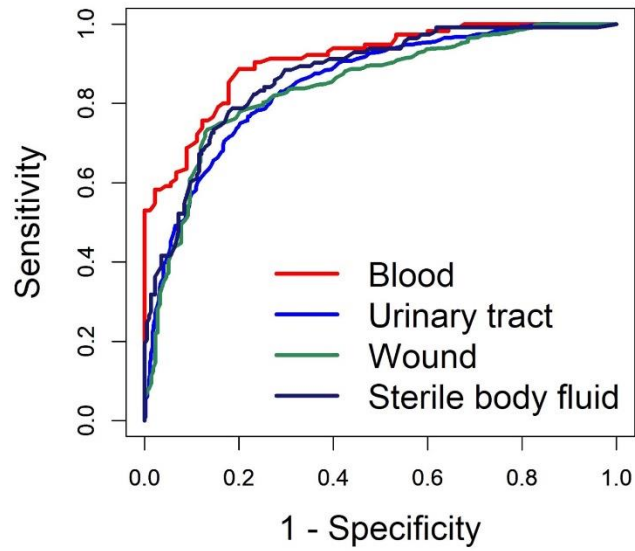


600

601 **Figure 3(c). ROC Curves for Different Algorithms in Terms of External Validation**

602

Figure 3(d)



603

604 **Figure 3(d). ROC Curves for the RF-Based VRE_{fm} Model With Different Types of**

605 **Specimens**

606

607 **Table 1. Performance of VRE_{fm} Prediction Models in Terms of k-Fold CV, Time-Wise**
 608 **Validation, and External Validation**

AUROC	RF	SVM	KNN
5-fold CV	0.8495 (0.8397, 0.8594)	0.8367 (0.8264, 0.8471)	0.7908 (0.7792, 0.8024)
Time-wise validation	0.8463 (0.8273, 0.8654)	0.8368 (0.8169, 0.8566)	0.7908 (0.7690, 0.8127)
External validation	0.8553 (0.8399, 0.8706)	0.8407 (0.8246, 0.8569)	0.8050 (0.7872, 0.8227)
Accuracy			
5-fold CV	0.7769 (0.7660, 0.7878)	0.7610 (0.7499, 0.7721)	0.7248 (0.7131, 0.7364)
Time-wise validation	0.7840 (0.7640, 0.8039)	0.7815 (0.7615, 0.8016)	0.7228 (0.7011, 0.7445)
External validation	0.7855 (0.7687, 0.8024)	0.7781 (0.7610, 0.7951)	0.7355 (0.7174, 0.7536)
Sensitivity			
5-fold CV	0.8054 (0.7951, 0.8517)	0.7826 (0.7719, 0.7934)	0.7873 (0.7767, 0.7980)
Time-wise validation	0.8153 (0.7965, 0.8341)	0.8415 (0.8238, 0.8592)	0.7491 (0.7281, 0.7702)
External validation	0.7791 (0.7620, 0.7961)	0.7954 (0.7789, 0.8120)	0.8044 (0.7881, 0.8207)
Specificity			
5-fold CV	0.7497 (0.7384, 0.7609)	0.7403 (0.7289, 0.7517)	0.6649 (0.6526, 0.6772)
Time-wise validation	0.7477 (0.7266, 0.7688)	0.7120 (0.6900, 0.7340)	0.6922 (0.6698, 0.7146)
External validation	0.7930 (0.7764, 0.8096)	0.7580 (0.7405, 0.7756)	0.6560 (0.6365, 0.6755)

609

610 AUROC, area under the receiver operating characteristic curve.

611

612 **Table 2. Performance of the RF-Based VRE_{fm} Detection Model With Different Types of**
613 **Specimens in Terms of External Validation**

Metrics	Type			
	Blood	Urinary tract	Sterile body fluid	Wound
AUROC	0.9103 (0.8727, 0.9480)	0.8494 (0.8258, 0.8731)	0.8714 (0.8321, 0.9106)	0.8432 (0.8121, 0.8743)
Accuracy	0.8488 (0.7997, 0.8978)	0.7743 (0.7482, 0.8004)	0.8077 (0.7657, 0.8497)	0.7740 (0.7436, 0.8043)
Sensitivity	0.8870 (0.8436, 0.9303)	0.7672 (0.7409, 0.7936)	0.7788 (0.7345, 0.8230)	0.7339 (0.7018, 0.7659)
Specificity	0.8000 (0.7452, 0.8548)	0.7805 (0.7547, 0.8063)	0.8222 (0.7815, 0.8630)	0.8676 (0.8430, 0.8922)

614

615