Reviewer #1 (Comments for the Author):  
  
The manuscript "Multi-omic 1 Signatures of Host Response Associated with Presence, Type, and Outcome of Enterococcal Bacteremia" addresses an important scientific question. It presents several interesting results. However, there are some flaws in analytical methodology which have to be addressed to ensure which results and more importantly which conclusions are well supported.

**Major comments**

1. Presently, individual features are identified as predictors of health vs. infection, E. Faecalis vs. E. Faecium, and survivorship vs. mortality. However, greater power could be achieved through a supervised machine learning approach, i.e., by constructing predictors that incorporate multiple factors, and the importance of individual factors could be determined from predictor composition. Moreover, training and evaluating a predictor on the same set of data can result in overfitting and an inaccurate estimate of predictor performance on new data. We therefore suggest the following:

1. For each of these comparisons, create one predictor or an ensemble of multiple predictors that utilize all markers passing some feature selection threshold.
2. Perform cross-validation on the training set after training to evaluate predictor performance within the training data.
3. Divide the samples into a training set and a test set. A commonly accepted ratio is 70% of samples in the training set and 30% of samples in the test set. Evaluate the predictor(s) on the test set to estimate predictor performance on new data. (Note: The test set would ideally be an entirely separate dataset. However, in the absence of such a dataset, this method allows predictor performance to still be estimated.)

2. It seems there is a design problem with comparison between infection and healthy individual. Control samples consist of plasma collected from blood bank volunteers, while EcB samples were collected from patients at UW Health. This discrepancy in patient and sample collection circumstances may be a significant source of confounding. Although steps are taken to identify confounding variables from patient metadata, it is nonetheless probable that further unidentified confounding variables remain.

There is potential solution at least partially overcome this problem. If we assume that the transition between health and infection shares a molecular basis with that between survivorship and mortality, then we would expect features truly caused by EcB to be shared between the two comparisons and to have the same direction (up or down) in both. We therefore suggest the following:

1. Create a contingency table which sets the two comparisons against each other.
2. If most features share a direction of change, use these features for the construction of predictors.
3. Otherwise, it is more likely that confounding variables will play a significant role in the infected/healthy analysis, and so it may be wiser to place the greater focus on the E. Faecalis vs. E. Faecium and survivorship vs. mortality analyses performed within the hospital dataset.

**Minor comments**

1. The sizes of the mortality and survival groups in Figure 1 do not sum to the same value as the E. Faecalis vs. E. Faecium groups (32 E. Faecalis + 44 E. Faecium = 76, while 17 mortality + 57 survival = 74). We suggest that this disparity be corrected if it is an error, or explained if it is not.
2. The subsection "Human Plasma Samples" under the section "Materials and Methods" states that data was collected from 32 patients with E. faecium bacteremia and 44 patients with E. faecalis bacteremia, totaling 76 patients (line 128). However, the following subsection "Clinical Data Collection", states that there were 83 patients with enterococcal bacteremia (line 137). We suggest that this disparity be corrected if it is an error, or explained if it is not.
3. In figure 1B, we suggest either placing the text in the title of each plot beneath its x-axis or removing the x-axis label to avoid duplication. Further, the title of the plot "Sensitivity to Vancomycin" is cut off.
4. On line 46, we suggest that the URL be made a hyperlink.
5. On line 46, the sentence is missing a period.
6. On line 134, a comma is missing after the closing paren.
7. On line 138, we suggest removing the comma after the word "identified".
8. On line 191, please check whether "cystines" should instead be "cysteines". It's possible that there may have been a misunderstanding on our end.
9. On line 199, we believe that the word "Analysis" should not be capitalized.
10. On line 208, The text "20oC" contains the character "o" rather than a degree symbol".
11. On line 211, the text "-80{degree sign}C{degree sign}C" contains a duplicate instance of "{degree sign}C".
12. Lines 218-221 use "minute" where they should say "minutes". We also suggest that semicolons are used to break up the list, such as "...following gradient: 0 to 1 minutes, 5% B; 1 to 7 minutes, a linear increase from 5% to 100% B; 7 to..."
13. On lines 233 and 234, the spacing around the equals signs is inconsistent.
14. On line 244, we suggest changing "GNPS FBMN option" to "the GNPS FBMN option".
15. On line 274, we believe "across any" should instead be either "for any" or "in any".
16. On line 281, a comma is missing after the word "randomforest".
17. On line 282, "R-package" should instead be "R package".
18. On lines 314 and 316, the temperatures "37C" and "50C" lack degree symbols.
19. On line 348, "GEForce" should instead be "GeForce".
20. On line 369, we suggest using "EcB" instead of "EB" for consistency.
21. On line 380, "Enterococcous" should instead be "Enterococcus".
22. On line 401, "1x1025" should instead be "1x10-25".
23. On line 417, "(30" should be "(30)".
24. On line 420, we believe the word "one" should be removed.
25. On lines 430 and 431, "gene ontology" should be capitalized to "Gene Ontology".
26. On line 461, a period is missing after the second close paren.
27. On line 484, there is an extra space before the em dash.

1. On line 512, the text "are leveraged" should be deleted.
2. On line 589, the word "of" should be inserted between the words "analysis" and "unsupervised".
3. On line 595, a period is missing after the close paren.
4. On line 662, a comma is missing between the words "biospecimen" and "and".

1. On line 758, a space is missing between the period and the word "Interestingly".

Reviewer #2 (Comments for the Author):  
  
The authors of this manuscript performed a comprehensive comparative analysis of plasma samples from 3 cohorts: 29 healthy volunteers, 32 patients with E. faecium bacteremia and 44 patients with E. faecalis bacteremia, with respect to the content of proteins and metabolites. Their main goals were to characterize the host response to Enterococcus bacteremia (EcB), to determine the systemic differences between E. faecalis and E. faecium bacteremia, and to identify the systemic response markers associated with increased mortality. They used an unbiased approach based on a comparison of proteoms and metaboloms of the collected plasma samples, supported by results of characterization of Enterococcus isolates from the patients and the selected case history parameters of each patient (including gender, the Charleston comorbidity index, duration of bacteremia, day of blood draw, and mortality during admission). Based on the used methodologies they could capture from among hundreds of proteins and metabolites analyzed, those that are increased or decreased in all or in particular types of bacteremias.   
They also compared their data concerning EcB with their earlier data concerning Staphylococcus aureus bacteremia. Based on the results of these comparisons they identified among detected plasma proteins and metabolites, the proteins and metabolized increased or decreased in bacteremia patients, proteins or metabolites common for all types of bacteremia, and proteins or metabolites with changed levels specific for E. faecalis, E. faecium or S. aureus bacteremia. Gene ontology (GO) enrichment analysis of proteins differentiating all or particular bacteremias allowed the authors to identify biological processes characteristically altered in all or particular kinds of studied bacteremias. Although the authors confirm the value of two previously approved markers for the detection of infection, most of their results are novel.   
The introduction section provides sufficient background and motivation to perform the studies described by the authors. The methods are described in sufficient detail. The results are mostly presented in the form of graphs that allows one to see the differences in plasma proteomes and metaboloms observed by the authors in infected versus healthy individuals. Statistical analysis is satisfactory. Additionally, the authors discuss the differences of insufficient statistical significance that can be potentially useful if further analyzed. The manuscript is a valuable contribution to the knowledge concerning common processes in the host response to all three kinds of bacteremias studied by the authors and processes in the host response characteristic for particular kinds of bacteremias. Additionally, it has a practical value as a guide to developing plasma analysis-based diagnostic methods that could be helpful in the fast detection of EcB cases, identification of the infecting Enterococcus species and the prediction of infection fatal outcome. An important observation of the authors was that most of the E. faecalis isolates were resistant to vancomycin, while vancomycin resistance among E. faecium isolates was rare, which may help in the fast decision concerning antibiotic therapy. In general, the manuscript is interesting to read**. Only the Discussion section seems to be too wordy, and could be shortened.**

My additional specific comments are below.  
  
L. 41. Please explain the abbreviations at their first use in the text

L. 311-312. Please provide a reference.

L. 312. "10"? What do you mean by that? Please explain.

L. 317. Please provide the catalog number.

L. 370. In the classification of patients with bacteremia, the authors took into account the duration of bacteremia. Could the beginning of persistent bacteremia be precisely determined by the authors? Please explain.

L. 380. Could the authors expect the detection of enterococcal proteins in blood samples? What was the range of titers of enterococci in patients' blood samples?

L. 561. With "E" ? Please provide a complete abbreviation.

L. 589-595. What was the reason of the differences between the initial and later findings?

Figure 3A. What do exactly the authors mean by "spectral match" and "no spectral match" in this Figure? This can be guessed intuitively. However, clear explanations by the author would be helpful for the readers.

Figure S2. Fonts in this Figure are too small to be readable. They should be at least slightly increased.

The authors compared their results with the previously described results obtained with similar methods for S. aureus bacteremia. In my opinion, the conclusions based on this kind of comparison should be made with care and used as suggestive rather than indicating something. The manuscript concerning staphylococcal bacteremia was published by the authors a few years ago. Thus, in the discussion section, the authors should at least briefly, compare the methodology of studies performed for that manuscript and the current manuscript.

The list of references requires extensive editing and supplementation with missing details concerning quite many listed publications.

L. 1022: Please provide the details of the cited publication

L. 1056. Does the reference cited apply to host-microbe interaction as suggested in the manuscript text? Please verify.

L. 1058, and elsewhere in the text: Please italicize genus and species names

L. 1090, 1092, and elsewhere in the text. Please supplement the list of references with multiple missing details.

Fonts in Figure S2 are too small and should be increased for better visibility.

Figures S8 and S9 are hardly readable. They should be provided in a better resolution.