

Author's response to reviews

Title: Emergence Of Unusual Species Of Enterococci Causing Infections, South India.

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Author's response to reviews: see over

Dear Sir/Madam,

Many thanks for considering our manuscript for publication in “BMC Infectious Diseases”. We are grateful to the reviewers for their authentic comments and suggestions that had really helped us to incorporate most of their suggestions in an appropriate manner. I hope we had tried to address all the suggestions/modifications as desired by the reviewers, and had given a point-by-point response as below mentioned.

Looking forward your kind response in this regard.

Yours sincerely,

Vittal Prakash.P

Pondicherry, INDIA.

I. Revisions made through modification/justification for Dr. Aristofanis Gikas’s comments.

1. In the Background section, the statement “Many studies focus on the two most common species *E. faecalis* and *E. faecium*, and only few reports or studies” should be cited with relevant references.

Modification: Relevant references [5,6] **have been cited in the background section** as suggested by the reviewer.

2. Please indicate the prevalence rates of common species of *E. faecalis* and *E. faecium*. I would suggest presenting the prevalence rates of all enterococci species in a relevant table.

Justification: We have **depicted** the prevalence of *E. faecalis* and *E. faecium* in the first paragraph of the results section **although indirectly** (i.e., It stands meaning that *E. faecalis* and *E. faecium* comprises the total of 242 enterococci excepting the 46 isolates [19%] of unusual species of enterococci depicted in this study). Further, we felt that stressing the prevalence of *E. faecalis* and *E. faecium* would dilute the objective of this study, which emphasizes the emergence of unusual species of enterococci in human infections.

3. Please indicate if there were any differences in the prevalence of unusual species by site of isolation and age.

Modification: The differences/breakup of 7 unusual species distributed among various sites **have been included in the first paragraph of the results section** as suggested by the reviewer.

4. In the Methods section, last sentence, first and second paragraph, the phrase “manufacturers instructions” should be corrected to “manufacturer’sTM instructions”. Also, the first sentence of Discussion section needs correction since the referring percentage of 19% could be misunderstood by the readers as a cumulative prevalence of unusual and atypical species.

Modification: a. The phrase “manufacturers instructions” **has been corrected** to “manufacturer’sTM instructions” in both the places as suggested by the reviewer.

b. Further, the first sentence of the Discussion section **has been corrected** as “Our study reveals that the prevalence of unusual species of enterococci as 19% in our clinical setup in South India” to make the statement clear as suggested by the reviewer.

II. Revisions made through modification/justification for Dr. Tsai-Ling

Lauderdale’s comments.

1. In Results section, since 2 of the 12 atypical strains were considered *E. casseliflavus*, why were they not counted with the 46 isolates?

Modification: The 46 unusual isolates includes the 2 atypical strains of *E. casseliflavus*, which was not mentioned mistakenly in the results. Hence as suggested by the reviewer **we’ve included the point** “The 2 (atypical) isolates of arginine negative variant *E. casseliflavus* like species after taxonomic validation were included as an unusual species of enterococci accounting to 3 *E. casseliflavus* isolated overall” **at the end of first paragraph of the results section** and had **included the fact in the results section of the Abstract.**

2. What is the species distribution of these 7 unusual species from blood and other sterile and non-sterile body sites?

Modification: The breakups of 7 unusual species distributed among various sites **have been included in the first paragraph of the results section** as suggested by the reviewer.

3. Some pertinent patient histories would help to highlight the clinical importance of these isolates.

Please refer to next point for the modification.

4. Since your study was performed over a 2-year study period, what is the time distribution of these 7 isolates? Was there an increase of isolation rate or clustering of a specific species? Did you see any nosocomial spread of these species?

Modification for comments **3 and 4**: As suggested by the reviewer **we have included the data regarding clinical importance and clustering of specific species, their prevalence and nosocomial spread** during our study period as follows **in the first paragraph of the discussion section**: "... Apart from septicemia, the unusual species of enterococci were isolated frequently from cases of urinary tract infections, surgical and non-surgical wound infections and peritonitis. Most of the patients (65%) with the bloodstream infections had a peripheral or central catheter..." **and** "... Although the unusual species of enterococci were isolated at regular intervals throughout our study period, we could find clustering of specific species during a specific time period from specific units/wards. Interestingly, 10 among the 15 isolates of *E. gallinarum* isolated during our study period were from pediatrics unit, while 7 of the 10

isolates exhibiting a similar antibiotype were isolated from the same ward within a span of 2 months. The remaining 3 of the 10 *E. gallinarum* were isolated from the same ward in the preceding 3 months, one of which showed an antibiotype similar to the cluster of 7 isolates. The same was the case of 3 *E. casseliflavus* isolated from the same pediatrics unit within a span of 2 months in the preceding year. Most of these (8 of 10 *E. gallinarum*, and all 3 *E. casseliflavus*) isolates were from cases of septicemia. Although molecular epidemiological studies have not been done to compare the genetic similarities of these isolates, the data depicts the nosocomial spread of these species.....”.

5. For antimicrobial susceptibility testing, list the antibiotics tested by disk diffusion (i.e., cipro, vanco), agar dilution in Materials section. Then make a table of the susceptible rates for each antibiotic by species (please arrange the species in alphabetical order instead of the way they are listed in Table 1). The way the MIC data is presented in Table 1 is confusing. Rather than listing how many isolates grew at each antibiotic concentration, list MIC range and % S for the agar dilution tested ones. There are some discrepancies in Table 1 also. For example, why is vancomycin susceptibility interpretation NA for agar dilution on some species? Since you also tested vancomycin by disk diffusion, you can list the vancomycin results by three methods in the table to show their discrepancy. In addition, why was BHI used instead of MH for disk diffusion and agar dilution? What QC organisms were used?

Modification: a. The list of the antibiotics tested by disk diffusion and agar dilution **have been included** as follows “... penicillin (10units), ampicillin (10 µg), gentamicin-high content (120 µg), streptomycin-high content (300 µg), ciprofloxacin (5 µg), nitrofurantoin - for urinary isolates only (300 µg), vancomycin (30 µg), teicoplanin (30 µg) and linezolid (30 µg)...”, **in the antimicrobial susceptibility testing sub-heading** under the Methodology section as suggested by the reviewer. Further, we have followed the NCCLS guidelines in performing the antimicrobial susceptibility testing with appropriate Q.C guidelines, and as per their guidelines as well based on literatures we have used BHI agar instead MH agar for our experiments [reference 9]. In a pilot study by our laboratory (data not shown) we found that BHI agar was equally good and supported luxuriant growth of enterococci making interpretations of AST results much easier and faster.

b. The species in Table-1 **have been arranged in alphabetical order** as suggested by the reviewer, although we haven't made a separate table as suggested by the reviewer for the susceptible rates for each antibiotic by species for the disk diffusion testing. We felt separate table was not required since the results of disk diffusion testing were in concordance with the results of MIC testing (depicted in Table-1) except vancomycin as explained in the results section.

c. We have presented the MIC data in Table 1 as per SENTRY antimicrobial surveillance format [reference 19]. We adapted this format since the listing of the number of isolates that grew at each antibiotic concentration would help us in categorizing/characterizing the antimicrobial resistant isolates, especially those isolates which exhibit intrinsic resistance to vancomycin (*E. gallinarum* and *E. casseliflavus*) posing difficulty in interpreting the results, as well other species exhibiting intrinsic resistance to various antibiotics.

d. Regarding the reviewers comment on vancomycin susceptibility interpretation as NA for agar dilution on some species in Table 1: We have depicted in Table-1 that results are NA-not applicable for 2 species *E. gallinarum* and *E. casseliflavus* since these 2 species exhibit intrinsic low level resistance for vancomycin (van C genotype), and still it is a debatable issue as whether to include the intrinsic low level resistance of these species as true resistance or not. Further, the interpretation of vancomycin resistance among these 2 species has always been a problem for most of the clinical microbiology laboratories worldwide, while some laboratories use PCR for species identification as well detecting the intrinsic resistance (van C) among these 2 species to resolve this issue [reference 1, 3, 5].

6. Please check reference carefully.

Modification: The references **have been checked** and **corrections have been made** appropriately as suggested by the reviewer.

III. Apart from the modifications for the reviewer's comments/suggestions, minor corrections have been made to the manuscript as depicted below;

1. The last sentence **in the Results section of the Abstract has been punctuated** as follows “..... vancomycin resistance by some species, while all the species tested were susceptible for linezolid and teicoplanin”.

2. The computational dendrogram of the gel image (Figure-1) was revised after analysis using Dice coefficient, instead of Pearson's coefficient as per standard procedures [4,10].