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P0081 Limited sensitivity and enrichment bias of culture-based pathogen detection in intensive care unit environment revealed by metagenomic sequencing

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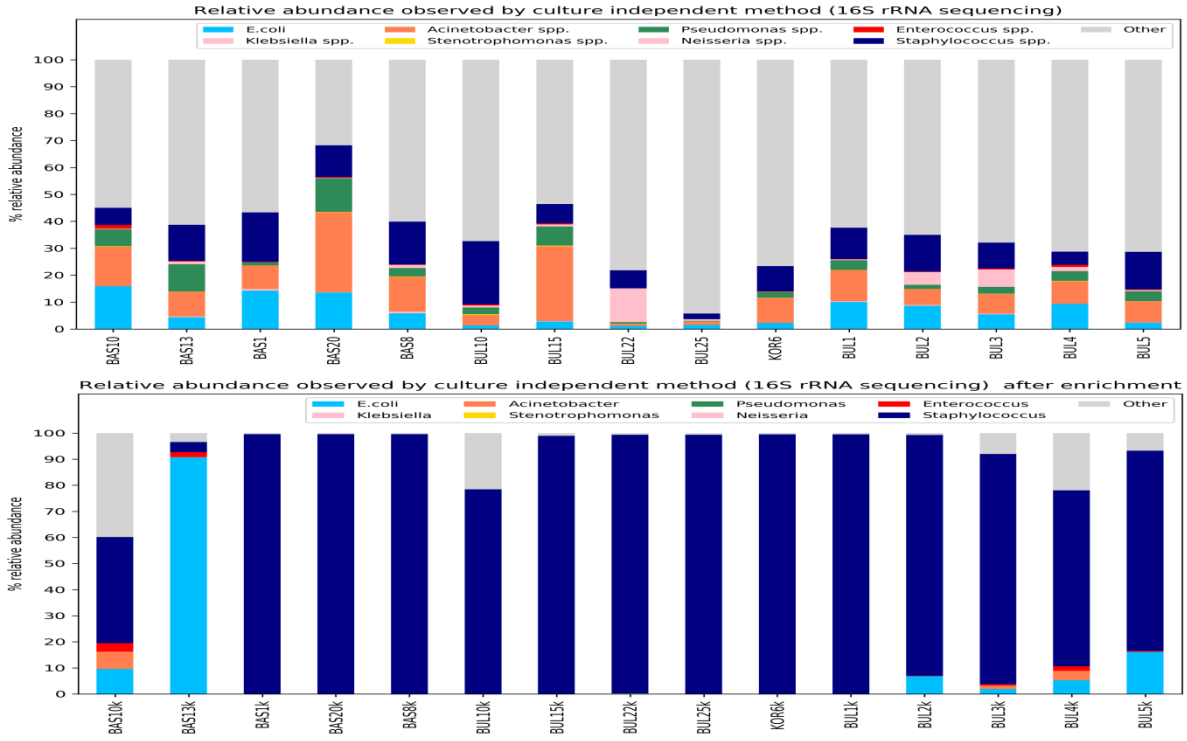
Background: The ability of microorganisms to survive on various surfaces in the hospital environment for a long time , as well as being resistant to certain antibiotics, and sometimes to antiseptics, constitute a serious risk for nosocomial infections. Culture-dependent investigation of environmental flora/pathogens typically consist of surface sampling, culturing, and identification steps. Next-generation sequencing, as a culture-independent methodology, is able to profile the taxonomic diversity belonging to the samples isolated from the surfaces. We investigated the sensitivity of culture-based environmental analysis by direct sequencing surface samples from Intensive Care Unit (ICU) environment, sequencing after enrichment, and comparing the results with the culture-based identifications.

Materials/methods: 15 surface samples from the isolation room of an adult ICU were obtained using triple sampling by sterile swabs. For each sample, culturing and identification, culture-independent 16S rRNA sequencing and taxonomic profiling were performed both directly from the sample and after enrichment. For direct culturing, the swab samples grown in EMB and in sheep blood agar. For culturing after enrichment, the samples were incubated in TSB for 24 hours, and then grown in EMB and in sheep blood agar. Commercial kits were used to isolate DNA from the samples before and after enrichment. Conventional identification and Vitek automatized analyzer identification was performed for cultured samples. The isolated microbial DNA was sequenced using 16S rRNA sequencing protocol in Illumina MiSeq system. QIIME taxonomic analysis pipeline was used to analyze the sequencing data.

Results: Bacterial growth was observed and identified in all enriched samples and 11 direct culturing samples predominantly detecting *Staphylococcus* species. 16S rRNA sequencing results indicate that although a diverse microbiota (total 202 genera), including pathogens of interest is included in the samples, the enrichment selectively amplifies a narrow phylogeny (total 31 genera detected after enrichment). While the enrichment emphasize *Staphylococcus* species, it was seen that some pathogens are eliminated (Figure below).

Conclusions: According to our results, while culturing directly from the environment can misdetect important pathogens in the ICU environment, culturing after enrichment might involve significant biases, implying an issue of pathogen detection sensitivity.


Relative abundance observed by culture independent method (16S rRNA sequencing)
E.coli
Klebsiella spp.
Acinetobacter spp.
Stenotrophomonas spp.
Pseudomonas spp.
Neisseria spp.
Enterococcus spp.
Staphylococcus spp.
Other
% relative abundance
0
10
20
30
40
50
60
70
80
90
100
BAS10
BAS13
BAS1
BAS20
BAS8
BUL10
BUL15
BUL22
BUL25
KOR6
BUL1
BUL2
BUL3
BUL4
BUL5

Relative abundance observed by culture independent method (16S rRNA sequencing) after enrichment
E.coli
Klebsiella
Acinetobacter
Stenotrophomonas
Pseudomonas
Neisseria
Enterococcus
Staphylococcus
Other
% relative abundance
0
10
20
30
40
50
60
70
80
90
100
BAS10K
BAS13K
BAS1K
BAS20K
BAS8K
BUL10K
BUL15K
BUL22K
BUL25K
KOR6K
BUL1K
BUL2K
BUL3K
BUL4K
BUL5K