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Paper Poster Session
Diagnosing gastrointestinal tract infection

Molecular tools for early detection and control of spread of multidrug resistant organisms in the healhcare setting

Icaro Boszczowski*[1], Maristela P. Freire[2], Thais Guimaraes[3], Patricia Dantas Da Silva Rebelo[4], Laura Maria Brasileiro Gomes[4], Isabel Crsitina Vilela Soares Oshiro[4], Ana Natiele Da Silva Barros[4], Dellyana Rodrigues Boberg[5], Marcos Oliveira de Carvalho[5], Luiz Felipe Valter de Oliveira[5], Anna Sara Levin[6]

[1]*Hospital Alemão Oswaldo Cruz and Hospital Das Clínicas Da Faculdade de Medicina Da Universidade de São Paulo, Infection Control Department, São Paulo, Brazil*

[2]*Instituto Do Cancer Do Estado de São Paulo, Infectious Diseases and Infection Control, São Paulo, Brazil*

[3]*Hospital Das Clínicas Da Faculdade de Medicina Da Universidade de São Paulo, Infection Control Department, São Paulo, Brazil*

[4]*Hospital Das Clínicas Da Faculdade de Medicina Da Universidade de São Paulo, São Paulo, Brazil*

[5]*Neoprospecta Microbiome Technologies, Florianópolis, Brazil*

[6]*Hospital Das Clinicas Da Faculdade de Medicina Da Universidade de São Paulo, Infectious Diseases, São Paulo, Brazil*

Background: Early detection of colonization by MDR pathogens is effective to control their spread. Results of surveillance cultures may take up to 5 days. Genomics and molecular biology enable identification of microorganisms and genes of resistance quickly and accurately. The objective of this study is to determine whether molecular methods (MM): genome sequencing (GS) and polymerase chain reaction (PCR), are sensitive to detect carriers of carbapenem resistant *Enterobacteriaceae* (CRE).

Material/methods: We performed a prospective cohort study during six weeks involving intensive care units in which surveillance cultures are routinely done for all patients on admission. Two rectal swabs were obtained, one for classical microbiology and another for MM. All patients whose admission cultures were negative were subjected weekly to the same routine. The performance of MM in identifying CRE was compared to classical microbiological.

Amplification and sequencing: were performed by Neoprospecta Microbiome Technologies and consisted in the rRNA 16S V3/V4 region amplification using the 341F (CCTACGGGRSGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) primers, with Illumina adapters. The amplification was performed in 35 cycles at 50ºC of annealing temperature, in triplicate. The sequencing was performed in Illumina MiSeq, using V2 kit, with a single-end 300nt run. Bioinformatics analysis: only sequences

with 275nt or more were used in downstream analysis. Read quality filter was performed converting Qscore in error probability, calculated by "Error probability" = 10^(-Qscore/10) for each nucleotide, and only reads with the sum of errors equal to or less than 1, were considered for downstream analysis. Then, all reads with one or more indeterminate bases "N" and truncated sequences with two or more consecutive bases with quality scores below to Q20, were eliminated. OTU Picking was performed using Blastn 2.2.28 against GreenGenes 13.8 database.

Results: 80 pairs of swabs were evaluated. Twenty-five (31.3%) yielded CRE by classical culture, 92% of them were *Klebsiella pneumoniae*. GS identified 800 species and 2,239,205 sequences. We found a 100% correspondence between GS and positive cultures in terms of species. Among culture negative swabs, in 98% were positive for *K.pneumoniae* when sequenced. Considering all samples sent to MM, polymerase chain reaction found bla_{KPC} in 18(22.5%), $bla_{CTX\text{-}M1}$ in 27(33.8%), $bla_{CTX\text{-}M9}$ in 7(8.8%), $bla_{OXA\text{-}23}$ in 10 (12.5%), $bla_{OXA\text{-}51}$ in 15 (18.8%). Genes $bla_{OXA\text{-}48}$ and bla_{NDM} were not found. Among 25 CRE obtained by culture, 15 detected bla_{KPC} by PCR and no bla_{NDM} or bla_{OXA48} were detected. Reliability inter-rates as measured by *Kappa* test was 0.59 (p<0.0001)

Conclusions: PCR led to rapid detection of resistance genes, which could enable rapid measures to prevent their spread. Patterns of sequenced fecal microbiota and their relationship to the acquisition and persistence of resistance genes require further studies.