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

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Background

Early detection of colonization by MDR pathogens is effective to control their spread. Surveillance cultures results take up to 5 days[1,2]. 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 at 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: this step was performed by Neoprospecta Microbiome Technologies. It 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: primers and adapters sequences were trimmed from the reads, and 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. To attribute taxonomy, only sequences with hits of 99% of identity were considered.

Results

We obtained 80 pairs of swabs during the study period. Twenty-five (31.3%) swabs yielded CRE by classical culture, 92% of them were *Klebsiella pneumoniae*. GS identified 800 species and 2,239,205 sequences. We found a 100% of correspondence between GS and positive cultures in terms of species. Among negative culture swabs, 98% of correspondent swabs sequenced *Klebsiella pneumoniae*. 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 amplified (table 1). Agreement among methods are shown in tables 2.

Table 1. Genes identified by polimerase chain reaction during prospective rectal surveillance in four intensive care units at a major teaching hospital in São Paulo, Brazil. August-September 2015 (n=80)

Gene	n (%)
bla_{KPC}	18(22.5)
$bla_{CTX\text{-}M1}$	27(33.8)
$bla_{CTX\text{-}M9}$	7(8.8)
$bla_{OXA\text{-}48}$	Not found
bla_{NDM}	Not found

Table2. Agreement among 80 pairs of rectal swabs comparing classical microbiology results yielding carbapenem resistant *Enterobacteriacea* and polimerase chain reaction detecting carbapenemases genes.

Classical culture *versus*	Liability inter-test	*Kappa*
KPC	.591	<0.001
CTXM	.421	<0.001
OXA23	.130	0.17
OXA51	.151	0.15
KPC+CTXM	.528	<0.001
TOTAL CARBAPENEMASES	.455	<0.001

Conclusions

PCR gives us a prompt answer whether the patient harbors a resistance gene enabling rapid measures to prevent their spread. Patterns of sequenced fecal microbiota and their-resistant genes are to be explored and may be a useful tool.

References

1. *Niederman MS. Impact of antibiotic resistance on clinical outcomes and the cost of care. Crit Care Med. 2001;29:114-20.*

2. Shwaber MJ, Carmeli Y. Carbapenem-resistence *Enterobacteriaceae*: a potential threat. JAMA. 2008;300(24):2911-2913