**Abstract**

Infections by Enterobacteriaceae resistant to carbapenems and/or carbapenemase producers emerging as an important challenge in the context of health care (Schwedter et al, 2008). Since the first report in 1999, North Carolina, USA, a plasmidic carbapenemase embedded in the chromosome of Enterobacteriaceae. This enzyme is strictly plasmid and confers resistance to all cephalosporins and monobactams, and reduced sensitivity to carbapenems in Enterobacteriaceae. Since the first report in 1999, North Carolina, USA, a plasmidic carbapenemase embedded in the chromosome of Enterobacteriaceae. This enzyme is strictly plasmid and confers resistance to all cephalosporins and monobactams, and reduced sensitivity to carbapenems in Enterobacteriaceae.

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**Introduction and Purpose**

Infections by Enterobacteriaceae resistant to carbapenems and/or carbapenemase producers emerging as an important challenge in the context of health care (Schwedter et al, 2008). Since the first report in 1999, North Carolina, USA, a plasmidic carbapenemase embedded in the chromosome of Enterobacteriaceae. This enzyme is strictly plasmid and confers resistance to all cephalosporins and monobactams, and reduced sensitivity to carbapenems in Enterobacteriaceae. Since the first report in 1999, North Carolina, USA, a plasmidic carbapenemase embedded in the chromosome of Enterobacteriaceae. This enzyme is strictly plasmid and confers resistance to all cephalosporins and monobactams, and reduced sensitivity to carbapenems in Enterobacteriaceae.

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**Methods**

**Samples**

We analyzed 156 rectal ESwab™ samples from patients hospitalized at the Hospital with suspected colonization with KPC-producing Enterobacteriaceae in the period from 15 March to 4 April 2010.

**Extraction of DNA**

Rectal swab samples were performed from 200 µl liquid ESwab™, using the QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer’s instructions.

**Real-Time PCR**

For real-time PCR reaction internal control was used 16S rRNA gene following the primers: sense 5’ATGCAAGTCGAGCGAAC3' and antisense 5’CCACATCTGGCTTGAAATTCTACTG3’. The reactions were performed in a final volume of 25 µl containing 1.5 µl SYBR Green I Master (PerkinElmer, California), 0.75 µl (10 µl containing 12.5 nM 5’GATGACCAGCTGTTCGTGTTC3' and antisense 5’CCACATCTGGCTTGAAATTCTACTG3’. The reactions were performed with a final concentration of 1.5% deoxyribonucleic acid (DNA) and 1.5 µl DNA from each rectal sample collected in liquid ESwab™ (QIAGEN, USA). Methods: The limit of detection (LOD) and cutoff were evaluated using positive and negative control according to GLSI documents EP-17, EP-12. The clinical sensitivity and specificity were calculated in a ROC curve using rectal swab samples from 156 patients hospitalized with suspected colonization by enterobacteria producing carbapenemase KPC during an clinical outbreak in 2010 at the São Paulo University Hospital. The 16S RNA gene was used as internal control.

Bacterial DNA was extracted using 20µl of liquid ESwab™ using the QIAamp DNA Mini Kit (QIAGEN, California) and amplification of gene KPC was analyzed in the real-time PCR using the Platinum SYBR Green qPCR Kit Super Mix (Invitrogen, CA, USA) and 7500 Real-Time PCR System equipment (Applied Biosystems, CA, USA). Results: The Cycle Threshold (Ct) (determined as LOD and cutoff of molecular assay were 36.7 and 37.6 respectively). The clinical sensitivity and specificity were 100% and 97.5% respectively with a threshold value of 18.9% for positive signal and one negative signal for each probe. Conclusion: These results suggest that real-time PCR for rectal swab direct detection for gene mKPC in Enterobacteriaceae can be useful in identifying patients colonized with bacteria producing carbapenemase KPC specialty for control of nosocomial outbreaks.

**Results**

The Real-Time PCR for gene KPC directly proved to be an excellent method for detection of reduced susceptibility to carbapenems in Enterobacteriaceae. Only one sample obtained inadmissible results due to failure of internal control by the probable presence of PCR inhibitors. The Real-Time PCR direct ESwab™ is useful in the investigation of patients colonized by Enterobacteriaceae producing KPC gene, which contributes to the control of hospital outbreaks.

**Conclusions**

The Real-Time PCR for gene KPC directly proved to be an excellent method for detection of reduced susceptibility to carbapenems in Enterobacteriaceae. Only one sample obtained inadmissible results due to failure of internal control by the probable presence of PCR inhibitors. The Real-Time PCR direct ESwab™ is useful in the investigation of patients colonized by Enterobacteriaceae producing KPC gene, which contributes to the control of hospital outbreaks.

**References**

Centers for Disease Control and Prevention. Guidelines for Control of Infections with Carbapenem-Resistant Enterobacteriaceae. EID-加快对药物的敏感性，避免患者的滥用，以及提高微生物检测的准确率。2015:20.(5)

Schwedter D, Naht T, Oettl S, Muth D, Hennig JC. Rapid detection of KPC-producing Enterobacteriaceae is of great importance in infection control and in controlling the spread of these microorganisms. The application of molecular methods in clinical samples requires analytical and clinical validation. J. The aim of this study was to perform the analytical and clinical validation of real-time PCR for rapid detection of gene mKPC from direct rectal sample collected in liquid ESwab™ (QIAGEN, USA). Methods: The limit of detection (LOD) and cutoff were evaluated using positive and negative control according to GLSI documents EP-17, EP-12. The clinical sensitivity and specificity were calculated in a ROC curve using rectal swab samples from 156 patients hospitalized with suspected colonization by enterobacteria producing carbapenemase KPC during an clinical outbreak in 2010 at the São Paulo University Hospital. The 16S RNA gene was used as internal control. Bacterial DNA was extracted using 20µl of liquid ESwab™ using the QIAamp DNA Mini Kit (QIAGEN, California) and amplification of gene KPC was analyzed in the real-time PCR using the Platinum SYBR Green qPCR Kit Super Mix (Invitrogen, CA, USA) and 7500 Real-Time PCR System equipment (Applied Biosystems, CA, USA). Results: The Cycle Threshold (Ct) (determined as LOD and cutoff of molecular assay were 36.7 and 37.6 respectively). Figure 3 shows the ROC area, and the clinical sensitivity and specificity were 100% and 97.5% respectively. The limit of detection (LOD) and the cutoff were 36.7 and 37.6 respectively. Figure 3 shows the ROC area, and the clinical sensitivity and specificity were 100% and 97.5% respectively.