[1]王慧娟,何云燕,夏云,等.多重耐药鲍曼不动杆菌gyrA基因高效反义肽核酸序列筛选及其体外抗菌活性观察[J].第三军医大学学报,2013,35(14):1442-1446.

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# 多重耐药鲍曼不动杆菌gyrA基因高效反义肽核酸序列筛选及其体外抗菌活性观察(PDF)

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Title: Screening of effective antisense peptide nucleic acids targeting gyrA from multidrug-resistant *Acinetobacter baumannii* and their antimicrobial effects *in vitro*

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关键词: 多重耐药鲍曼不动杆菌; gyrA; 反义寡核苷酸; 斑点杂交; 肽核酸

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摘要: 目的 筛选出能与多重耐药鲍曼不动杆菌gyrA基因的mRNA紧密结合的反义肽核酸序列，评估其体外抗菌活性。 方法 利用Mfold、RNA Structure 4.6两种计算机软件对多重耐药鲍曼不动杆菌gyrA基因的mRNA进行二级结构分析计算，根据最低自由能原则在其mRNA局部杂交松弛区设计反义寡核苷酸，与体外转录的地高辛标记的gyrA mRNA进行斑点杂交，根据杂交信号的强弱筛选出与gyrA mRNA紧密结合的反义寡核苷酸，根据其序列合成肽核酸(peptide nucleic acid，PNA)，另加

设1条与靶序列有6个碱基错配的PNA作为阴性对照，2条PNA分别连接穿膜肽形成肽-肽核酸(peptide-PNA，PPNA)。测定经不同浓度PPNA处理的细菌光密度[*D*（600）]值并进行平板菌落计数，观察其对细菌生长的抑制作用，采用RT-PCR检测gyrA mRNA的表达水平。　结果　斑点杂交结果显示，计算机软件设计的10条反义寡核苷酸探针中有5条与gyrA mRNA显示出杂交信号，其中1条信号最强，能够与gyrA mRNA稳定结合，将其以肽-肽核酸的形式合成处理细菌，结果表明其在5 μmol/L浓度可完全抑制gyrA mRNA的表达，并抑制鲍曼不动杆菌的生长，在10 μmol/L浓度具有杀菌活性，而具有错配碱基的PPNA对细菌生长无明显抑制作用。　结论　计算机辅助设计联合斑点杂交可在体外模拟体内环境对寡核苷酸与靶基因结合的有效性进行验证，实现在体外高通量筛选高效反义序列。经筛选得到的靶向gyrA基因反义肽-肽核酸可在体外高效抑制多重耐药鲍曼不动杆菌生长。

Abstract: Objective To screen the effective antisense peptide nucleic acids targeting gyrA gene from multidrug-resistant *Acinetobacter baumannii*, and to evaluate their antimicrobial effects *in vitro*. Methods Two RNA folding computer programs, Mfold and RNA structure 4.6, were used to predict the secondary structure of gyrA mRNA, and then 10 antisense oligonucleotides were designed based on free energy theory. The full length of gyrA mRNA was transcribed *in vitro* and labeled by digoxigenin-11-uridine-5′-triphosphate. Dot blot hybridization was used to screen the gyrA mRNA accessible sites which showed strong binding affinity to the antisense oligonucleotides. Peptide nucleic acid (PNA) was synthesized based on the sequence of antisense oligonucleotide showing high affinity. Another PNA oligomer containing 6 mismatched nucleotides was used as a negative control. Both the 2 PNAs were conjugated to cell penetrating peptide (CPPs)（KFF）K to form peptide-PNA (PPNA). After the