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Global Perspective of CR Elements Associated with Metallo-ß-Lactamase Producing Isolates: Report from the SENTRY Antimicrobial Surveillance Program

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Background: The metallo-ß-lactamase (MßL) gene bla_{SPM-1} is associated with the genetic element CR4, a novel transposable structure. Similar elements (CR1) have also been identified with several antibiotic genes such as bla_{CMY-9}, $bla_{CTX-M-2}$, $bla_{CTX-M-9}$, *catA2*, *dfr*A10. CR2 is found as part of an SXT-type transposon from *Vibrio cholera*. In this report, we screened all of the SENTRY MßL producing isolates and examined the linkage of MßL genes with CR elements.
Methods: Degenerate primers were designed against an alignment of the four CR elements that have been described so far and used to amplify similar regions from target strains. In addition, primers designed against bla_{VIM} genes and the CR elements were used in PCR reactions to detect whether the MßL genes were in the vicinity of these elements.
Results: PCR products of expected size (800bp) were produced in all SPM-1 producing strains from Brazil. In addition, positive PCR products of identical size were also produced for VIM-1 producing strains 62-2633, 62-5149 isolated in Greece, VIM-1 producing strains 85-2966, 85-4744 isolated in Sicily, VIM-2 producing *Acinetobacter* strains 82-5139, 82-5935 recently identified in Germany and VIM-7 producing strain 07-406 from the USA. PCR reactions performed to detect the proximity of MßL genes to CR elements produced products <2kb with the Greek bla_{VIM-1} containing isolate 62-2633 and German bla_{VIM-2} containing strains 82-5139 and 82-5935.
Conclusions: CR elements are found in strains containing the MßL gene bla_{VIM-1} from Greece and Italy, bla_{VIM-7} from USA and bla_{VIM-2} isolated in Germany. The CR element was found to be in close association with the MßL gene in a Greek strain and two German *Acinetobacter* strains. The close association between MßL genes and this putative transposable structure indicates that it is very likely to be involved in the dissemination of MßL genes.

INTRODUCTION

Class 1 integrons that are defective transposon derivatives have been divided in three main groups depending on their backbone structure. These are the In4, In5 and In6 families. Common Region (CR) elements were initially identified in the In6 family as a section of DNA that was common to In6 and In7, later designated CR to distinguish it from the conserved sequences (CS's) of the integrons. In the In6 and In7 family integrons, the CR element is found downstream of both the variable region (containing the gene cassettes) and the 3'CS of the integrons. In both integron families the CR element is associated with an antimicrobial resistance gene that is not in the form of a gene cassette but lies immediately adjacent to it. The genes are *dfr*A10 in In7 and *catA2* in In6. A second truncated copy of the 3'CS of the integron is found further downstream of these resistance genes.

The CR elements were initially classified in CR1, CR2 and CR3 based on their DNA sequence. Alignments of the CR elements of various integrons and the 3' sequences adjacent to the antimicrobial genes associated with them revealed a similar sequence at the 3' end of all CR elements. It is thought that this short sequence is likely to be recognized by the product of the CR gene which may be a recombinase involved in the incorporation of antimicrobial resistance genes. More recently a new CR element, CR4, was found adjacent to the newly identified bla_{SPM-1}, and it may constitute a tool for its dissemination. The bla_{SPM-1} is unique among metallo-ß-lactamase (MßL) genes as not being found in the form of a gene cassette. In this study we characterize the CR elements of all MßL producing isolates collected by the SENTRY Antimicrobial Surveillance Program, and also evaluated any possible linkage between the CR elements and the MßL genes.

METHODS

Bacterial Isolates: The SENTRY Program monitors pathogen frequency and antimicrobial resistance patterns of nosocomial and community-acquired infections through sentinel hospitals worldwide (1997-date). Among other selected pathogens, *Pseudomonas* spp. and *Acinetobacter* spp. strains resistant to imipenem (MIC, ≥ 16 µg/ml), meropenem (MIC, ≥ 16 µg/ml), and ceftazidime (MIC, ≥ 32 µg/ml) have been routinely examined for antimicrobial resistance genes since January 2001 through the amplification and sequencing of the class 1 integrons. The entire SENTRY Program collection of MßL-producing isolates was screened with CR primers in the present study. MßL-producing isolates collected by the BCARE (University of Bristol, UK) in some European countries, including Germany and Poland, were also evaluated.

Primer design: Representative members of each CR family (CR1-4) were aligned and degenerate PCR primers were designed to anneal to sections which showed high identity between all family members. These primers shown below were designed to anneal at positions 962 and 1737 of the nucleotide alignment and should amplify an internal CR section of approximately 780 bp.

CRF: CACTWCCACATGCTGTKKC
CRR: CGCTTGAGSCGTTGCRYCC

Primers were also designed to determine if the MßL genes were closely linked to the CR elements, which may indicate their involvement in the gene dissemination. These primers were the complimentary sequence of the above CR primers along with primers anchored to the various MßL genes.

PCR conditions. The PCR reaction was performed using 1 µl of an OD 1 at 600 nm suspension as template and involved initial denaturing for 5 minutes at 95°C followed by 30 cycles of annealing, extension and denaturing steps of 1 minute each at temperatures of 50°C, 68°C (3 minutes for extended PCRs) and 95°C, respectively and a final step of extension at 68°C for 5 minutes.

RESULTS

Presence of CR elements in MßL-producing isolates.

- PCR amplicons were produced from the following strains:
  - IMP-1-producing *Acinetobacter* spp. strain 48-5227 and SPM-1-producing *Pseudomonas aeruginosa* strains 101-1629 and 48-25 from Brazil.
  - VIM-1-producing *Acinetobacter baumannii* 62-2633 and *P. aeruginosa* 62-5149 from Greece and *P. aeruginosa* strains 85-2966 and 85-4744 from Sicily.
  - VIM-2-producing *A. baumannii* 82-5139 and 82-5935 from Germany.
  - VIM-4-producing *P. aeruginosa* 303/03 from Poland.
  - VIM-7-producing *P. aeruginosa* 07-406 from the USA.

Alignments of CR elements.

- The CR element from the Brazilian IMP-1-producing isolate 48-5227 was found to be very similar to CR2 previously found to be associated with florphenicol resistance genes (Figure 1).
- Deduced protein sequences of the CR elements amplified from isolates 85-2966, 85-4744, 101-1629, 48-25, 82-5139, 82-5935 and 62-5149 were all aligned with CR3 (Figure 2).
  - The CR elements from the Italian VIM-1-producing isolates were 100% identical to CR3.
  - The CR element from the SPM-1-producing isolates that were sequenced was 90% identical to CR3.
  - The CR elements from the German VIM-2-producing isolates and the Greek VIM-1-producing isolate were identical to each other yet shared only 79% identity to CR3.
- The section of the CR element amplified from the VIM-7-producing isolate (USA) was found to be 91% identical to CR3 (Figure 3).
- The CR elements of the SPM-1-producing Brazilian isolates 101-1629 (Brasilia) and 48-25 (Sao Paulo) were both 89% identical to CR3, but only 75% identical to CR4 (previously identified in a SPM-1-producing isolate from Recife, Brazil [Poirel et al., 2004]).

Linkage of CR elements.

- PCR amplification using primers anchored to the CR elements and the respective MßL genes within individual isolates determined that the CR element in German VIM-2 producing isolates 82-5139 and 82-5935 was approximately 1.8kb downstream of the bla_{VIM-2} and that the CR element in the Greek VIM-1-producing strain was approximately 1.5kb downstream of the bla_{VIM-1} (data not shown).
- Analysis of the American VIM-7 producing isolate 07-406 revealed that the CR element in this strain was not closely associated with bla_{VIM-7}, but was located immediately upstream of bla_{OXA-45}.
- Similarly, the CR element was located immediately upstream of bla_{SPM-1} in all SPM-1-producing isolates.

Figure 1. Alignment of deduced protein sequence of the CR of isolate 48-5227 with CR2 previously found in *P. damsalae*, *E.coli* and *K.pneumoniae* strains associated with the florphenicol resistance gene *floR*. The CR element of the IMP-1 producing strain 48-5227 shares ~99% identity with CR2 through the amplified section.

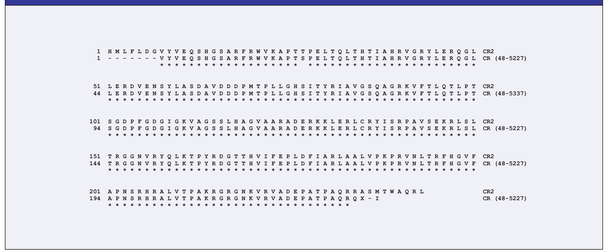


Figure 2. Alignment of amplified regions of CR elements from MßL producing isolates with the common region of CR1 identified upstream bla_{PSE-1} in the MDR region of Salmonella genomic island 1. Asterisks below the alignment represent residues which are identical in all CRs. CR elements amplified from Italian strains 85-2966 and 85-4744 producing the VIM-1 MßL were 100% identical to CR3. The CR element from the SPM-1 producing isolate 101-1629 was 90% identical to CR3. The CR from the VIM-2 producing *Acinetobacter* spp. strains from Germany and the VIM-1 producing *P. aeruginosa* strains from Greece were 79% identical to CR3.

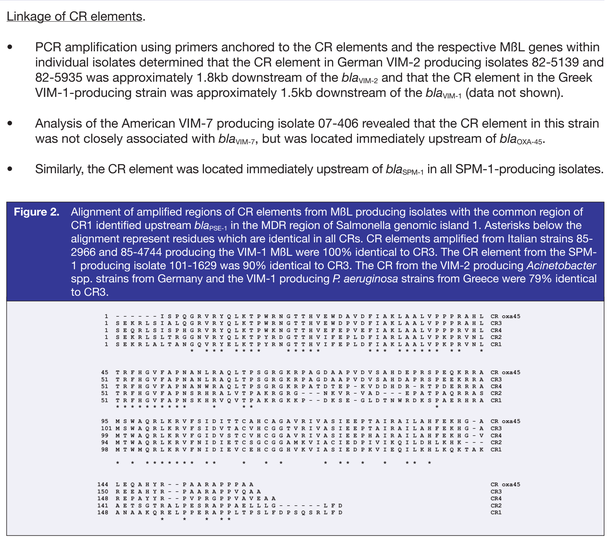
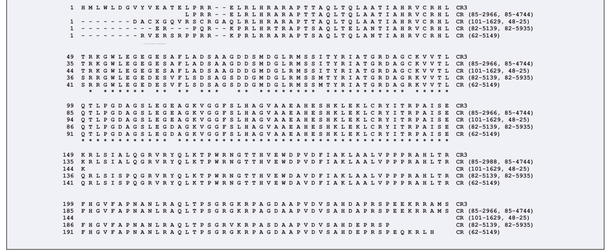


Figure 3. Alignment of the C-terminal portion of the CR found closely associated with the bla_{OXA-45} gene in the VIM-7 producing isolate 07-406. The portion of the CR is aligned with the protein sequences of CR1-4 and displayed most identity to CR3. Percent identities were 91%, 74%, 52% and 51% to CR3, CR4, CR1 and CR2, respectively. Residues that were identical in all CRs are indicated with asterixes.



CONCLUSIONS

- The differences found between the CR elements of the bla_{SPM-1} gene of the two *P. aeruginosa* strains from Brazil suggest that there are more than one CR element in these particular strains or that bla_{SPM-1} can be associated with different CR elements. Work is presently continuing on the linkage of the CR elements to bla_{SPM-1} in all SENTRY Program SPM-1 producing isolates.
- The CR elements harboured by VIM-2-producing strains (82-5139 and 82-5935) from Germany and VIM-1-producing strain 62-5149 from Greece were considerably different from CR3 and probably constitute a new type of CR element (CR5).
- The finding of the CR element downstream of the bla_{VIM-1} in the German isolates and in the Greek isolate, and immediately upstream of the bla_{OXA-45} in the American strain (07-406), suggests that these CR elements are involved in the mobility of MßL genes.

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