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Carbapenem-Resistant *Serratia marcescens* Producing SME-2, a Carbapenem-Hydrolyzing Class A ß-Lactamase, Isolated in Houston, Texas: Report from the SENTRY Antimicrobial Surveillance Program (2004)

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AMENDED ABSTRACT

Background: Since the report of SME-1 in London, rare SME-type enzyme (Group 2f) producing *S. marcescens* strains have been isolated from Minnesota, California, Massachusetts and Illinois over a period of 20 years. We characterized two carbapenems (CARB)- R *S. marcescens* strains recently isolated in Houston, Texas, through the 2004 SENTRY Program.

Methods: The antimicrobial susceptibility (S) profile of the strains against > 30 agents was determined by CLSI broth microdilution methods. The production of metallo-ß-lactamase was screened by disk approximation test and Etest (AB BIODISK, Solna, Sweden) MßL strips. Imipenem (IMP) hydrolysis rates were obtained spectrophotometrically. The characterization of $\mathit{bla}_{\text{SME}}$ was carried out by PCR using genetic primers followed by DNA sequencing. The isolates were epidemiologically typed by automated ribotyping and PFGE.

Results: The strains were isolated from bloodstream infections in February and August, 2004. The strains were S to ceftriaxone, ceftazidime and cefepime (MICs, ≤ 0.25 µg/ml), intermediate to aztreonam (MIC, 16 µg/ml), and R to IMP, meropenem and ertapenem (MIC, > 32 µg/ml). The strains showed significant CARB hydrolysis at comparable rates. The PCR utilizing generic primers for the $\mathit{bla}_{\text{SME}}$ family amplified a 650-bp amplicon with sequencing results consistent with $\mathit{bla}_{\text{SME-2}}$ (GenBank AB 275256).

Conclusions: We report the emergence of SME-2-producing *S. marcescens* in a USA geographic region where this enzyme has not been previously reported. Physicians should be cautious when prescribing CARB empirically assuming that *S. marcescens* isolates found to be S for broad-spectrum cephalosporins would also be S to CARB, this is not true for the Group 2f ß-lactamases.

INTRODUCTION

Carbapenems are ß-lactam antimicrobial agents with broad antibacterial activity, increased stability to ß-lactamase hydrolysis, and high rates of penetration through the bacterial outer membrane. Most of ß-lactamases capable of hydrolyzing carbapenems belongs to Ambler molecular class B, also named metallo-enzymes. Many types of metallo-ß-lactamases, such as IMP-, VIM-, SPM-, GIM-, and SIM, have been isolated mainly from non-fermentative Gram-negative bacilli, but also less commonly among the Enterobacteriaceae (IMP and VIM type).

SME-1 is a serine carbapenemase first identified from imipenem-resistant *S. marcescens* strains in London (1982), before clinical approval of carbapenems. This chromosomal enzyme belongs to class A ß-lactamases and is capable of hydrolyzing penicillin, aztreonam, and older cephalosporins in addition to imipenem. Since the discovery of SME-1, four sets of imipenem-resistant *S. marcescens* strains have been isolated from distinct regions of the United States: i) Minnesota (1985); ii) California (1992); iii) Boston (1994-1999), and iv) Chicago (1998).

SME-2 has a single substitution in the deduced amino acid sequence compared to the original SME-1 (Y for I at position 245). A modified enzyme, SME-3, was found in strains collected in Boston, California, and Chicago. The strains from Boston and California showed three nucleotide substitutions compared to SME-2: A to G at 258, A to T at 620 and A to G at 714, resulting in a single amino acid change, E for V at position 207. Purified SME enzymes show similar biochemical profiles. In the present study, we characterized two carbapenem-resistant *S. marcescens* strains recently isolated in Houston, Texas in 2004 by the SENTRY Antimicrobial Surveillance Program.

MATERIALS AND METHODS

Bacterial Strains and Susceptibility Testing. As part of the SENTRY Program, two *S. marcescens* isolates (24-1068A and 24-8089A) showing carbapenem resistance were forwarded to the monitoring laboratory. The strains were isolated from the bloodstream of patients hospitalized in a medical center located in Houston, Texas. At the monitoring laboratory, antimicrobial susceptibility testing was performed using the reference broth microdilution method as described by the Clinical Laboratory Standards Institute. Quality control was performed by testing *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922.

Carbapenemase screening. Members of the Enterobacteriaceae family showing decreased susceptibility (MIC, ≥ 2 µg/ml) to imipenem or meropenem are routinely screened for metallo-ß-lactamase production by disk approximation test and Etest MßL strips according to the manufacturer's instructions (AB BIODISK, Solna, Sweden). Screening for serine carbapenemases is performed by disk approximation test using clavulanic acid as an inhibitor.

Carbapenem hydrolysis. The *S. marcescens* isolates were grown in Nutrient Broth with ceftazidime (4 µg/ml), pelleted, resuspended in 1 ml of 1mM TRIS, 100 mM $ZnCl_2$ solution and then ultrasonicated. The cell extract was centrifuged and 100 µl of the supernatant was used to carry out the spectrophotometer assay against imipenem at 299 nm. MßL-inhibition was performed at room temperature by incubating the protein extract for 15 minutes with 25 mM EDTA and then assayed against imipenem.

PCR experiments. $\mathit{bla}_{\text{IMP}}$, $\mathit{bla}_{\text{VIM}}$ and $\mathit{bla}_{\text{SPM}}$ were initially screened by PCR amplification using primers designed for the internal fragment of the MßL genes. The detection of the MßL related genes was carried out in 20µl final volume using ABgene Taq DNA Polymerase (ABgene House, Surrey, United Kingdom). Primers were used at 10 mM concentration and 1µl of overnight bacterial culture at OD 1 at 600 nm was used as a template. The cycling parameters were: 95°C for 5 minutes followed by 30 cycles of 95°C for 1 minute, annealing at 45°C for 1 minute followed by extension at 68°C for 1 to 4 minutes and ending with 5 minute incubation at 68°C. The characterization of $\mathit{bla}_{\text{SME}}$ was carried out by PCR using generic primers followed by DNA sequencing.

DNA sequencing and computer analysis. The amplicons obtained by PCR reactions were sequenced on both strands using ABIPrism 377 system. The nucleotide sequences were analyzed using Lasergene software package (DNASTAR, Madison, WI). Obtained sequences were compared to sequences available over the internet (http://www.ncbi.nlm.nih.gov/blast/ and http://www.ebi.as.uk/fasta33/).

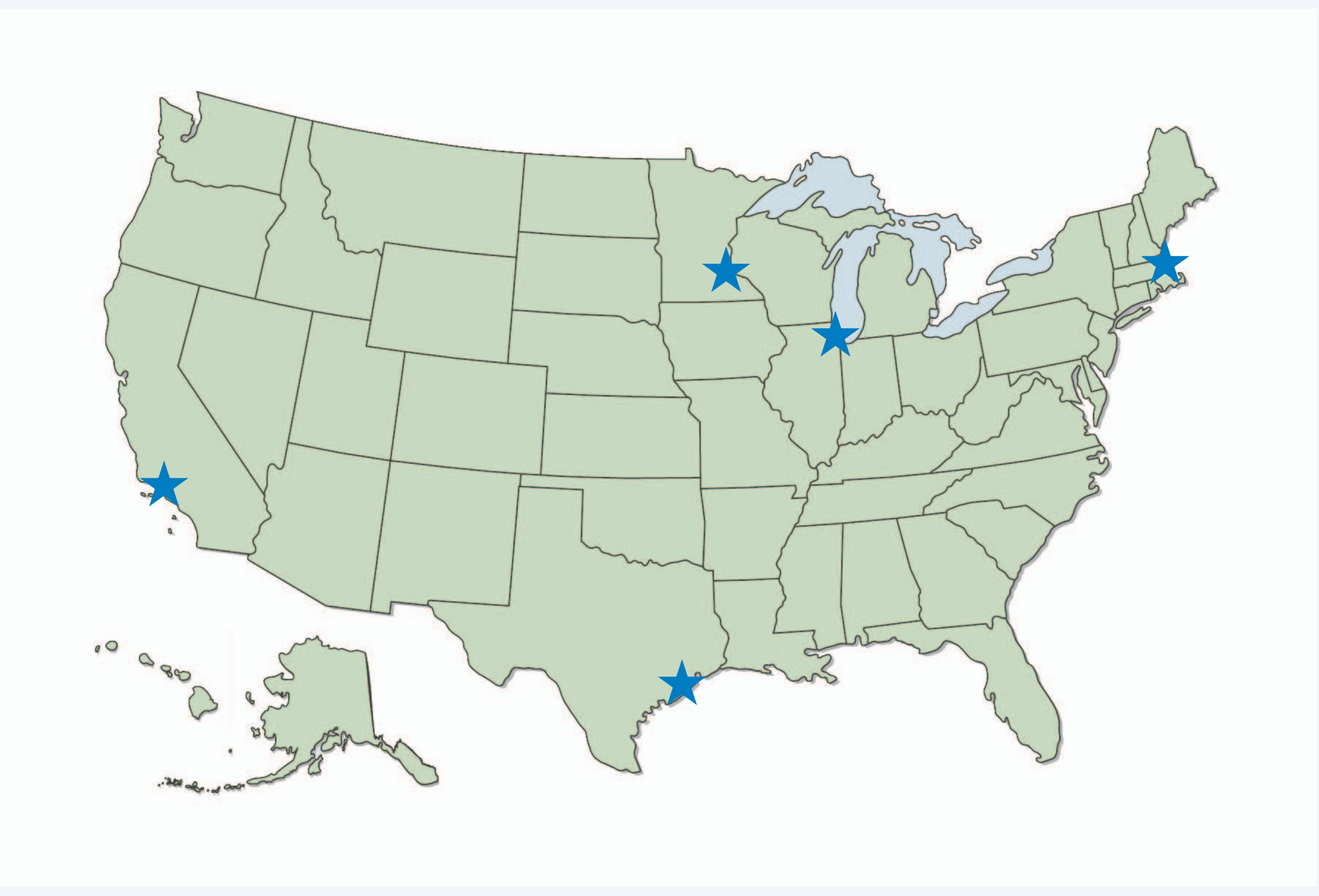
Molecular Typing. The *S. marcescens* isolates were molecular typed by ribotyping using the RiboPrinter™ Microbial Characterization system (E.I. duPont de Nemours, Wilmington, DE) according to the manufacturer's instructions. Isolates exhibiting coefficient of similarity ≥ 0.93 are grouped under the same ribogroup.

RESULTS

- The strains were susceptible to ceftriaxone, ceftazidime and cefepime (MICs, ≤ 0.25 µg/ml), intermediate to aztreonam (MIC, 16 µg/ml), and resistant to imipenem, meropenem and ertapenem (MIC, > 32 µg/ml).

- No enhancement of the diffusion zones was observed between the EDTA and imipenem or ceftazidime disks. In addition, there was no decrease in the imipenem MIC value in the presence of EDTA. Thus, results of both phenotypic MßL screen tests were negative. Clavulanic acid inhibition tests were positive.

Figure 1. US locations where SME-producing *S. marcescens* isolates have been described.



- Both *S. marcescens* strains showed strong imipenem hydrolysis at comparable rates.

- The imipenem hydrolysis was not inhibited by EDTA, indicating no production of MßL. In addition, no PCR product was obtained with primers for MßL genes.

- The PCR utilizing generic primers for the $\mathit{bla}_{\text{SME}}$ family of enzymes amplified a 650-bp amplicon with sequencing results consistent with $\mathit{bla}_{\text{SME-2}}$ (GenBank AB 275256).

- The strains showed distinct ribogroups, indicating no epidemiologic link.

Figure 2. Alignment of SME-2 (GenBank AB275256) with protein fragments from DNA sequences obtained from strains 24-1068A and 24-8089A.

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24-8089A - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - 0
24-1068A - - - - - - - - - - - - - - - - - - - - - - - - - - - N K S 3
SME-2    M S N K V N F K T A S F L F S V C L A L S A F N A H A N K S 30

24-8089A - - - - - - - - - - - - - - - G R I G V F A I D T G S G N T 15
24-1068A D A A A K Q I K K L E E D F D G R I G V F A I D T G S G N T 33
SME-2    D A A A K Q I K K L E E D F D G R I G V F A I D T G S G N T 60

24-8089A F G Y R S D E R F P L C S S F K G F L A A A V L E R V Q Q K 45
24-1068A F G Y R S D E R F P L C S S F K G F L A A A V L E R V Q Q K 63
SME-2    F G Y R S D E R F P L C S S F K G F L A A A V L E R V Q Q K 90

24-8089A K L D I N Q K V K Y E S R D L E Y H S P I T T K Y K G S G M 75
24-1068A K L D I N Q K V K Y E S R D L E Y H S P I T T K Y K G S G M 93
SME-2    K L D I N Q K V K Y E S R D L E Y H S P I T T K Y K G S G M 120

24-8089A T L G D M A S A A L Q Y S D N G A T N I I M E R F L G G P E 105
24-1068A T L G D M A S A A L Q Y S D N G A T N I I M E R F L G G P E 123
SME-2    T L G D M A S A A L Q Y S D N G A T N I I M E R F L G G P E 150

24-8089A G M T K F M R S I G D N E F R L D R W E L E L N T A I P G D 135
24-1068A G M T K F M R S I G D N E F R L D R W E L E L N T A I P G D 153
SME-2    G M T K F M R S I G D N E F R L D R W E L E L N T A I P G D 180

24-8089A K R D T S T P K A V A N S L N K L A L G N V L N A K E K A I 165
24-1068A K R D T S T P K A V A N S L N K L A L G N V L N A K E K A I 183
SME-2    K R D T S T P K A V A N S L N K L A L G N V L N A K E K A I 210

24-8089A Y Q N W L K G N T T G D A R I R A S V P A D W V V G D K T G 195
24-1068A Y Q N W L K G N T T G D A R I R A S V P A D W V V G D K T G 213
SME-2    Y Q N W L K G N T T G D A R I R A S V P A D W V V G D K T G 240

24-8089A S C G A Y G T A N D Y A V I W P K N R A P L - - - - - - - - 217
24-1068A S C G A Y G T A N D Y A V I W P K - - - - - - - - - - - - - 230
SME-2    S C G A Y G T A N D Y A V I W P K N R A P L I V S I Y T T R 270

24-8089A - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
24-1068A - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
SME-2    K S K D D K H S D K T I A E A S R I A I Q A I D - - - - - - 294
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CONCLUSIONS

- This study shows the emergence of SME-2-producing *S. marcescens* in a Texas hospital, a distinct USA geographic region, where this enzyme had not been previously reported.

- According to CLSI guidelines, carbapenems should be tested and reported selectively for Enterobacteriaceae isolates. However, SME-producing *S. marcescens* may show susceptibility to broad-spectrum ("third- or fourth-generation") cephalosporins and resistance to carbapenems.

- Physicians and microbiologists alike, should be aware of the emergence of this resistance phenotype and should not assume that cephalosporin-susceptible Enterobacteriaceae are carbapenem-susceptible as well.

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