The Emergence of the Extended-Spectrum ß-Lactamase GES-1 in a *Pseudomonas aeruginosa* strain in Brazil: Report from the SENTRY Antimicrobial Surveillance Program

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AMENDED ABSTRACT

Background: Guiana Extended-Spectrum (GES)-1 is an extended-spectrum ß-lactamase (ESBL) recently described in *K. pneumoniae* in France from a patient previously hospitalized in the French Guiana. To date, reports of this ESBL have been restricted to European countries.

Methods: As part of the SENTRY Program, all isolates are susceptibility tested by NCCLS broth microdilution to >30 antimicrobials. Strains resistant to imipenem, meropenem and ceftazidime have been screened for antimicrobial resistant genes. Isolates were screened using PCR with sets of primers specific to the 5'CS and 3'CS of Class 1 integron. The PCR products were sequenced bidirectionally using DuPont Automated systems and the results analyzed by DNAstar.

Results: Strain 48-8896 from Sao Paulo, Brazil was a *P. aeruginosa* isolated from blood in a 63 yo female who was admitted for a hysterectomy due to endometrial sarcoma. Seven days after surgery, the patient developed a wound infection while receiving cephalothin, amikacin and metronidazole. The first-generation cephalosporin was replaced by ceftriaxone, and later all antimicrobials were empirically replaced by polymyxin B and vancomycin due to clinical deterioration. The patient died after 3 months in the ICU. Strain 48-8896 showed high-level resistance to all antimicrobial agents evaluated, except polymyxin B (MIC, ≤1 μg/ml). PCR results for bla_{IMP}, bla_{VIM} and bla_{SPM} were negative. Sequence analysis revealed the ESBL GES-1. Upstream from the ESBL lies a Class 1 integron and downstream lies a gene cassette *cat*B8.

Conclusions: This is the first report of the GES-1 ESBL outside of Europe. The SENTRY Program has detected the regional emergence and the worldwide dissemination of several ß-lactamases now including GES-1.

BACKGROUND

GES-1 was initially isolated from a *Klebsiella pneumoniae* strain in 1998. Although the GES-1 producing-*K. pneumoniae* strain had been isolated in a French hospital, it was isolated in the first day of admission from a patient transferred from Cayenne, French Guiana, South America. The patient was a newborn, who had been hospitalized in Cayenne for treatment of a neonatal infection due to *Morganella morganii*. Following three weeks of cefotaxime and netilmicin therapy, the patient was transferred to the intensive care unit (ICU) of the French hospital where a rectal swab was collected and the GES-1 producing-*K. pneumoniae* isolated.

After the leading report, bla_{GES-1} was also found in a *P. aeruginosa* strain isolated in another France hospital. In contrast to the resistant pattern observed in the *K. pneumoniae*, the GES-1 producing *P. aeruginosa* isolate showed a higher level of resistance to ceftazidime than to ticarcillin, and synergy between the ß-lactamase inhibitors and broad spectrum cephalosporins was not detected. An outbreak of *K. pneumoniae* harboring GES-1 was recently reported in a hospital in Lisbon, Portugal. Twenty-four isolates from different sources were identified carrying GES-1 in diverse molecular size plasmids.

As part of the SENTRY Antimicrobial Surveillance Program, selected multi-drug resistant strains are screened for antimicrobial resistance genes. In this report, we describe the bla_{GES-1} found in a *P. aeruginosa* strain isolated in Brazil.

CASE REPORT

Patient MSS, a 63 year-old white female, presented with a 3-month history of uterine cramps, metrorrhagia, and weight loss. An endometrial biopsy was performed through curettage and revealed endometrial sarcoma. On May 16th, 2002 the patient was admitted for a hysterectomy at Hospital Sao Paulo, Sao Paulo, Brazil. Seven days after surgery, the patient developed a wound infection while receiving cephalothin, amikacin and metronidazole. The first-generation cephalosporin was then replaced by ceftriaxone. Amikacin and metronidazole were kept along with ceftriaxone for an additional 14-day period, when the patient showed clinical deterioration and was transferred to the ICU. Blood culture was drawn and polymyxin B plus vancomycin were started empirically on June 10th. A multidrug-resistant *Pseudomonas aeruginosa*, strain 48-8896, grew two days later and this antimicrobial regimen was maintained for a total of 14 days. The infection was eradicated but the patient developed clinical complications and died after being hospitalized for 3 months in the ICU.

MATERIAL & METHODS

Susceptibility testing. Antimicrobial susceptibility testing was performed using the reference broth microdilution method as described by the National Committee for Clinical Laboratory Standards (NCCLS). Antimicrobial agents were obtained from the respective manufacturers and quality control was performed by testing *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212.

Phenotypic detection of ß-lactamases. Production of metallo-ß-lactamases was screened by the disk approximation test. Briefly, a 100mm Mueller-Hinton agar plate was inoculated using a 0.5 McFarland suspension from fresh cultures. Imipenem, meropenem, and ceftazidime disks were strategically aligned around disks contained either EDTA (750 μg) or thiolactic acid (0.3 μl). The test was read after 18-20 hrs of incubation at 35ºC. The appearance of either an elongated or a phantom zone between the carbapenems and/or ceftazidime and either one of the disks containing a metallo-ß-lactamase inhibitor (EDTA or thiolactic acid) was considered a positive test. *Acinetobacter baumannii* 54/97 was used as a positive control. MBL Etest® strips (AB Biodisk, Solna, Sweden) were used to confirm the disk approximation test results. In addition, ceftazidime/ceftazidime-clavulanic acid and cefepime/cefepime-clavulanic acid ESBL Etest® strips were used to evaluate the production of extended-spectrum ß-lactamase (ESBL). The Etest® strips were used according to the manufacturer's instructions.

Antimicrobial resistance gene screening. Among other selected pathogens, *P. aeruginosa* strains non-susceptible to imipenem (MIC, ≥ 16 μg/ml), meropenem (MIC, ≥ 16 μg/ml), and ceftazidime (MIC, ≥ 32 μg/ml) have been routinely examined for antimicrobial resistant genes through the amplification and sequencing of the variable region of Class I integrons. *P. aeruginosa* 48-8896 was grown overnight in nutrient agar with 20 μg/ml of ceftazidime at 37ºC. One loopful of bacterial growth was suspended in 300 μl of molecular biology grade water. One μl of this suspension was used as template in standard PCR conditions, with a low stringency annealing temperature (45ºC). Since several ß-lactamase genes are part of gene cassettes that are class 1 integron encoded and most of them contain an aminoglycoside acetyl transferase cassette, primers located in the 5' conserved segment region (5'-CS) (5'-CCAAGCTCTCGGGTAACATC-3') and in the flanking region of *aacA4* (5'-AACTTGCGAGCGATCCGATG-3') were used for PCR amplifications.

DNA sequencing. A amplicon of 1211 bp was sequenced in both directions using DuPont Automated systems. The nucleotide and amino-acid sequences were analyzed and compared to sequences available in web based databases by using the Lasergene software package (DNASTAR, Madison, WI).

Analytical IEF. The ß-lactamase extract from strain 48-8896 was obtained by cell lysis with BugBuster (Novagen, Nottingham, United Kingdom) and the experiment was performed with a NOVEX (Invitrogen, Paisley, United Kingdom) apparatus. The focused beta-lactamases were detected by overlaying the gel with nitrocefin solution (Microbiology Systems, Cockeysville, MD). Isoeletric points were estimated by linear regression obtained by comparison to reference proteins by using a pI 4.5 to 9.5 Standard IEF marker (Bio-Rad, Watford, United Kingdom).

Plasmid analyzes and transformation. Plasmid extraction was carried out with QIAprep Spin Mini prep kit (Quiagen, West Sussex, United Kingdom). Transformation was performed as previously described in *E. coli* DH5α and selected in nutrient agar plates with 4 μg/ml of ceftazidime.

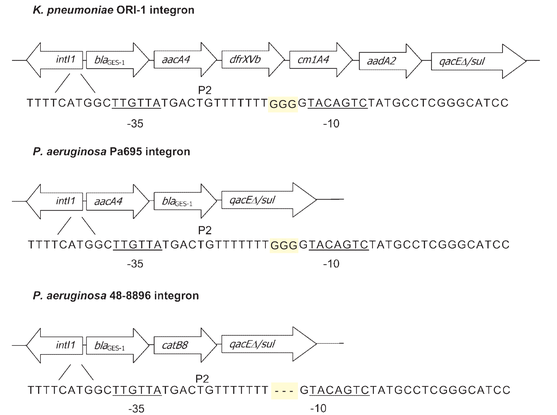
COMMENTS

- *P. aeruginosa* strain 48-8896 showed resistance to imipenem (MIC, >8 μg/ml), meropenem (MIC, >8 μg/ml), ceftazidime (MIC, >16 μg/ml), cefepime (MIC, >16 μg/ml), piperacillin/tazobactam (MIC, >64 μg/ml), and all antimicrobial agents evaluated, except polymyxin B (MIC, ≤1 μg/ml).
- Results of the disk approximation and Etest® methods were negative for detection of both metallo-ß-lactamases and extended-spectrum ß-lactamases.
- Sequence analyzes of the 1211 bp PCR product showed a bla_{GES-1} in the first position of an class 1 integron.
- The DNA fragment presented a similar adjacent and upstream context as described in the first report of GES-1 (*K. pneumoniae* ORI-1). Beyond bla_{GES-1} was *intI1*, encoding the integrase of a class 1 integron. Between bla_{GES-1} and the *intI1* was the *attI1* site and both promoters as detected in the strain ORI-1. However, the P2 promoter appears to have a 3 bp deletion, and the space between the -35 and -10 sequences is only 14 bp (Figure 1).

COMMENTS (continued)

- On the other side was the *catB8*, which encoded a chloramphenicol-acetyl transferase, followed by the 3'CS region typical of class 1 integron.
- IEF analysis showed that *P. aeruginosa* 48-8896 produced a ß-lactamase with pI value of 5.8, that corresponds to the GES-1.
- Plasmid DNA preparations did not show any plasmids, and transformation in *E. coli* was unsuccessful.
- The detection of a GES-1-producing *P. aeruginosa* isolate in Latin America (Brazil) is very worrisome since it raises the possibility for the emergence and future dissemination of new GES derivatives with broader spectrum of hydrolyses, such as GES-2, a carbapenem-hydrolyzing enzyme.

Figure 1. Schematic representations of the different bla_{GES-1} containing integrons. The 3 bp deletion found in the P2 of *P. aeruginosa* 48-8896 is highlighted. The genetic environment found in strain 48-8896 was very distinct from that found in the previously described GES-1 producing isolates.



CONCLUSIONS

- The genetic environment found in the integron of strain 48-8896 is remarkably different from that previously described in other GES-1 positive strains, suggesting that this gene cassette has moved between different organisms and that it may have widely disseminated.
- The finding of the bla_{GES-1} gene in the chromosome of a *P. aeruginosa* isolate causing infection in a Brazilian hospital may indicate the regional spread of this gene.
- Antimicrobial surveillance programs, such as the SENTRY Program, play an important role in detecting the emergence and dissemination of antimicrobial resistance mechanisms.

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