Carbapenems-resistant Pseudomonas aeruginosa bloodstream infection in Hematopoietic stem cell transplant patients: in vitro synergy and clinical outcome

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DOI: 10.21203/rs.2.12342/v1

SUBJECT AREAS
Infectious Diseases
KEYWORDS

Pseudomonas aeruginosa bacteraemia; multidrug resistance; virulence; in vitro synergy; transplant infection
Abstract
Summary

Bloodstream infection (BSI) caused by Pseudomonas aeruginosa has high mortality in hematopoietic stem cell transplant (HSCT) recipients. Objectives: To evaluate clinical, in vitro synergy and molecular features of 30 BSI caused by carbapenems-resistant P. aeruginosa (CRPA) in HSCT patients. Methods: Demographic and clinical data including treatment were collected and a database was built using EPIINFO, bivariate and multivariate analysis were run to assess outcome using SPSS. In vitro synergy using time-kill assays, pulsed-field electrophoresis (PFGE) and PCR for carbapenemases and virulence genes were performed for all isolates. Whole genome sequence (WGS) of main clones was done by Nextera XT, using Illumina MiSeq technology. Results: Mortality was 71%; most patients who died were allogeneic HSCT (78%) (p=0.02). Clinical isolates showed a high resistance level to meropenem: 50% had a MIC of 512 µg/mL; two thirds were susceptible to amikacin (MIC 2-512 µg/mL) and 100% to colistin. Many (17/30) isolates achieved synergistic effect with meropenem plus colistin but not with amikacin. None antagonism was observed. The presence of synergy by time-kill between colistin and meropenem showed a tendency towards a better outcome (HR 0.68 95%CI 0.02-1.02; p=0.06). The most frequent carbapenemase gene identified was blaSPM, and six co-harboured both blaKPC and blaSPM. Isolates presented genes related with virulence factors such as toxA, exoS and more patients with BSI caused by P. aeruginosa harbouring gene lasB evolved to death. The WGS showed that all five clones harboured SPM-1, Tn4371 and belonged to ST277; however, the resistance and virulence genes differ among the clones. Conclusion: CRPA BSI showed high mortality in HSCT patients. All clones carried SPM-1, Tn4371 and belonged to ST277. Synergy by time-kill between colistin and meropenem showed a tendency towards a better outcome

Background

Healthcare-associated infections (HAIs) are currently among the major challenges to the quality of patient care in hematopoietic stem cell transplantation (HSCT). Transplanted patients acquire HAIs due to impaired immunity, hospitalization, and use of invasive devices [1].

Bloodstream infection (BSI) caused by Pseudomonas aeruginosa has especially high morbidity and
mortality in this population of patients and its empirical treatment is obligatory during febrile neutropenia [1-3]. These bacteria can harbour multiple antibiotic resistance mechanisms, leading to limited therapeutic options [4]. Recently, during an outbreak in our hospital, we conduct a case-control study and observed that prior use of carbapenem was the only independent risk factor for carbapenems-resistant *P. aeruginosa* (CRPA) BSI (P=0.043) [5].

To date, few studies have evaluated antimicrobial combinations and *in vitro* synergy against CRPA [6-7].

In addition to resistance, the expression of several virulence genes by *P. aeruginosa* strains such as elastase and endotoxins, involved on tissue degradation and biofilm, has been associated with more severe infections and higher mortality [9-11].

We described *in vitro* synergy assays results in carbapenems-resistant *Pseudomonas aeruginosa* (CRPA) isolates, including clinical and microbiological data.

**Methods**

Thirty patients with BSI due to CRPA were included in the study conducted at the bone marrow unit of Hospital das Clínicas, a 1,900-bed, tertiary-care public teaching hospital, from December 2011 through December 2014.

**Patients’ characteristics**

Bloodstream infection diagnostic was based on Centers for Diseases Control and Prevention (CDC) criteria [12]. Data on epidemiological and clinical characteristics of infection and outcome of HSCT recipients were obtained from electronic medical records and Pitt bacteraemia score was calculated [13]. All transplanted recipients had a central venous catheter and received levofloxacin as antibiotic prophylaxis during neutropenia. Initial antibiotic therapy was considered appropriate when colistin (COL) was administered within 24 hours after obtain blood culture. All patients received 2g of meropenem (MERO) tid, prolonged infusions (3 hours) adjusted for kidney function when indicated. Amikacin (AMK) was added to the combination therapy with colistin and meropenem whenever CRPA was susceptible.
**Microbiologic assays**

Minimum inhibitory concentrations (MIC) of colistin (USP Reference Standard, Rockville, MD, USA), amikacin (Sigma-Aldrich, St Louis, MO, USA), and meropenem (Astra Zeneca, Cotia, SP, Brazil) were determined using the broth microdilution method in duplicate on separate days, according to the Clinical and Laboratory Standards Institute (CLSI) [14]. *Pseudomonas aeruginosa* ATCC27853 was used as control.

**In vitro synergy**

The synergistic effect was investigated by two methods checkerboard and Time-kill assays.

*Checkerboard assay*

The CRPA isolates were exposed to combination of two drugs, and checkerboard microdilution testing was performed in duplicate and evaluated after 20–24 hours of incubation at 35°C. Growth and sterility controls were tested in all plates. Colistin, meropenem and amikacin were combined at the respective minimum inhibitory concentrations determined by microdilution. The antimicrobial agents were diluted from the stock solution, and left at concentrations 4 times higher than the final concentration in plate, and then a serial dilution was performed. The results were interpreted using 2-well method, with synergy defined as the absence of growth in wells containing 0.25 x MIC of both drugs and 2 x MIC of both drugs [15].

*Time-kill assay*

Time-kill assay was performed in duplicate with drugs alone and combined at 1x MIC and 0.5x MIC as previously reported [16]. Flasks containing Mueller Hinton broth and the drug were inoculated with testing organisms at a density of ~10⁶ cfu/mL, a final volume of 10 mL, and incubated in a shaker at 35°C in ambient air. Aliquots were removed at time 0 and 2, 4, 6, and 24 hours’ post-inoculation and serially diluted in 0.85% sodium chloride solution. Diluted samples of 0.01 mL were plated in duplicate on Müller Hinton agar, and the colonies were counted (log₁₀ cfu/mL) after 20 hours of
incubation at 37°C. Synergism was interpreted as a $\geq 2 \log_{10}$ decrease in colony count with the antimicrobial combination compared to the most active single agent; the combination was considered antagonistic for a $\geq 2 \log_{10}$ increase in cfu/mL, and indifferent for a $< 2 \log_{10}$ increase or decrease in count with the combination compared with the most active drug alone [16-17].

**Resistance and virulence genes**
Polymerase chain reaction (PCR) for the carbapenemase genes, $bla_{SPM}$, $bla_{VIM}$, $bla_{NDM}$, and $bla_{KPC}$ and for virulence genes, $lasB$, $exoS$, $phZN$, $toxA$ and $ecfX$ which is intrinsic in *P. aeruginosa* were performed as previously described.\(^\text{18,19}\) The molecular profile was assessed by pulsed field gel electrophoresis (PFGE), using S-pel restriction enzyme (Fermentas, USA) in chromosomal DNA Ultrapure Agarose (Invitrogen, Life Technologies). Patterns were interpreted according to Bionumerics software version 7.1 (Applied-Maths, Sint-Martens-Latem, Belgium). A Dice coefficient $>0.80$ was considered the cut-off for potential clonal relatedness. The resistance and virulence genes, including $exoU$ gene, of five main clones were searched by whole-genome sequence (WGS) using Illumina MiSeq and Ion Torrent technologies. *De novo* assembly of reads was performed using Velvet Optimiser v.2.2.5.5 (Victorian Bioinformatics Consortium, Australia). Contigs were ordered by Abacas v.1.3.1 and Prokka v.1.11 performed genome annotation\(^\text{20,21}\). Multilocus Sequence Typing (MLST) (http://pubmlst.org/paeruginosa) investigated clonal relatedness. The sequencing reads of the isolates were mapped against *P. aeruginosa* PAO1 (Genbank accession number AE004091.2) using BWA, SAMtools and Genome Analysis Toolkit (GATK) to identify the Single nucleotide polymorphisms (SNPs).

**Statistical analysis**
A database was built using the Epi Info™ version 7.2. Chi-square or Fisher’s exact tests were used for categorical variables, and Mann–Whitney’s test for continuous variables, comparing patients’ characteristics, laboratory values, and treatments. Statistical analyses were performed using SPSS software version 21 (SPSS Inc., USA). Bivariate Cox regression was used to estimate Hazard Ratios
(HR) with the respective 95% confidence intervals. For variables with levels of significance lower than 0.2 (p<0.2) in bivariate tests, the Cox multiple regression was done. Seven-day and 14-day mortality were determined. A 2-tailed alpha less than 5% was considered significant for all statistical tests.

Results
Among 40 patients affected during entire study period, 30 were included since their bacterial isolates were available and feasible. Posteriorly two were excluded from clinical analysis because their HSCTs were postponed. They were mostly female (18/28); the median age was 42 years old. Acute leukaemia was the most frequent underlying disease (12/28), followed by lymphomas (6/28). A small proportion of patients have disease in full remission (6/28). Nineteen were allogeneic HSCT.

Seventeen of twenty-five were not colonized by CRPA prior to infection. The mean Pitt score for bacteraemia severity at the time of infection was 1.10; four patients (14%) had score > 4. The median length of stay before infection was 19 days. Twenty-six patients (93%) had neutropenia at the time of a positive blood culture; 14-Day mortality was 71% (20/28), 95% of them in the first seven days onset of CRPA-BSI (Table 1).

In vitro susceptibility tests of thirty clinical isolates showed a high proportion of resistance to meropenem: 50% (15/30) had a MIC of 512 ug/mL, 47% (14/30) a MIC of 256 ug/mL and one isolate had a MIC of 16 ug/mL. Sixty-six per cent were resistant to amikacin (MIC 2-512 ug/mL), and all of them were susceptible to colistin; moreover, the last four isolates presented higher colistin MICs (4.0 ug/mL). The most frequent carbapenemase identified was SPM, and six co-harboured both bla<sub>KPC</sub> and bla<sub>SPM</sub> genes. None carried bla<sub>VIM</sub> or bla<sub>NDM</sub> (Table 2).

The combination of COL plus MERO assessed by time-kill achieved synergy in 57% of isolates (17/30) of COL plus AMK in two of 30 isolates (6.6%), and of MERO plus AMK in 33% (10/30), only in isolates already susceptible to AMK. Of the antimicrobial combinations performed by checkerboard using two-well method interpretation the highest number of synergy was found between MERO plus AMK (9/30). There was no synergistic effect between COL and AMK. No antagonism was observed.

Regarding synergy and treatment outcome, twenty-five patients who received polimyxin-based therapy were analysed separately (Table 3). Most patients who died were allogeneic HSCT recipients
The presence of synergy by time-kill between colistin and meropenem showed a tendency towards to better outcome (HR 0.68 95%CI 0.02-1.02; 𝑝=0.06), which was not observe with the combination between meropenem and amikacin.

All five virulence genes searched were isolated in 21 isolates (70%) by PCR technique. Only the lasB gene was found in a lower proportion (83%). Mortality was higher in patients presenting CRPA carrying the lasB virulence factor gene (88.9% vs. 57.1%, HR 2.0 95%IC 0.39-10.5; 𝑝=0.11).

The WGS showed that all clones harboured SPM-1 (except the isolate CloneD/N14), Tn4371 and belonged to ST277 and none harboured exoU gene (Table 2).

The analysis revealed the presence of several genes related to resistance to aminoglycosides, beta-lactam, fluoroquinolone, phenicol and sulphonamide. Regarding virulence genes, all the strains harboured genes involved on quorum sensing, biofilm formation, adhesion and invasion process and cytotoxicity.

The study of outer membrane proteins, in all isolates, showed the presence of the same mutations on the porins OprD and OprE, and in parts of the efflux pumps presents in the strains: MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexGHI-OpmD. Mutations were also found on the post-transcriptional activator of the efflux pump MexEF-OprN.

Discussion

Our findings demonstrated a high mortality of CRPA-BSI in HSCT patients, and that death was more frequent among allogeneic patients, similar to those found in the literature [22]. In our cohort, however, Pitt bacteraemia score did not discriminate severe cases as observed in intensive care units [10]. Interestingly, prior gut colonization was also not common in this population, maybe due to low sensitivity of traditional culture methods to assess colonization or as an indication of cross-transmission as important source for HAI infection in our hospital5. Although, five clones were found, we observed a predominant clone, showing that cross–infection occurred during the study period.

Spite of fact of the strains were resistant to meropenem, half of them achieved in vitro synergy in combinations of meropenem with colistin, and the group that received this combination showed a tendency towards lower mortality (6 of 7 patients that survived received meropenem plus colistin).
Zusman et al (2013) performed a meta-analysis of 15 published studies and found similar results for in vitro combination of carbapenem and polymixin E in 59% (95% CI, 30 to 83%) among 43 CRPA isolates, although with variable methods and different carbapenem (doripenem, meropenem and imipenem)[5].

In contrast with previous studies [6], the combination with amikacin was not synergistic in our casuistic, and even in a small population of patients, this finding highlights possible harmful effects of this combination, such as nephrotoxicity, with no apparent benefit.

Combination therapy is tempting since it increases the likelihood of success in initial therapy, which is crucial in a population as vulnerable as neutropenic. Hu et al. (2013) conducted a meta-analysis of combination therapy versus monotherapy for P. aeruginosa bacteraemia included 10 clinical studies in a meta-analysis but did not find a mortality difference. Up to now, the best experimental results occurred with doripenem as part of in vitro combination [8], but the clinical experience with this drug in the HSCT population is scarce. Doripenem is not available in Brazil, thus, we could not evaluate it as an option to treat our patients [25-26].

The isolates harboured important virulence attributes such as exoS, toxA. Recently, a study showed an association between exoU gene expression and mortality among 590 patients with P. aeruginosa bacteremia. The exoU gene was not identified in our clones probably because they are multidrug resistant. We found that patients with BSI caused by isolated harbouring gene lasB evolved more often to death. This virulence factor is associated with bacterial enzyme production of elastase that degrades immunoglobulin and complement factor [27].

The most frequent carbapenemase identified was blaSPM; however, six co-harboured blaKPC and blaSPM. The blaSPM was located in the Tn4371, alerting for the potential of dissemination of this lineage in the world [28-34]. The strains accessed by WGS had mutations on porins OprD and OprE, and efflux pumps: MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexGHI-OpmD. Mutations were also found in the post-transcriptional activator of the efflux pump MexEF-OprN. Estepa et al (2017) described these mutations F170L, T103S and K115T in OprD protein in carbapenems-resistance P. aeruginosa.
The polymorphisms observed in the OMPs genes in our strains could be directly related to the resistance to carbapenems and explained the resistance found in the carbapenemase-negative strains in our casuistic.

Although, all clones belonged to ST277, which is common in our country and has been described in several outbreaks including patients who travelled to Brazil [29-34]. The WGS brought important information regarding resistance and virulence genes that differs among the clones. For instance, 16S rRNA methyltransferase gene \( \text{rmtD1} \), which confers high-level resistance to all aminoglycosides and has been associated to ST277 was present only in clones A, D and E. Clone C did not harbour \( \text{fos}A \); it carried \( \text{exoS} \) and \( \text{toxA} \) of Type Three Secretion System (TTSS) and lacked the \( \text{exoY} \) gene. The analyses also confirm this large number of virulence factors such as \( \text{exoS} \) and \( \text{exoY} \), as well as quorum sensing, biofilm and genes of phenazine operons that are responsible for increasing intracellular oxidative stress [8,25].

There are few studies of CRPA-BSI especially in such a homogeneous patient population. However, the small number of patients is an important limitation of our study.

Conclusions
The CRPA-BSI had high mortality in HSCT patients and even higher among allogeneic patients. Pitt bacteraemia score did not discriminate severe cases as observed in intensive care units. Treatment using combination of meropenem with colistin, showed a tendency towards lower mortality and displayed high \textit{in vitro} synergy. Further investigation using antibiotic combination treating a large population is essential to confirm our findings.

Abbreviations
AMK: amikacin; BSI: Bloodstream Infection; COL: colistin; CRPA: carbapenem-resistant \textit{Pseudomonas aeruginosa}; HSCT: hematopoietic stem cell transplantation; MERO: meropenem; MLST: Multilocus Sequence Typing; PFGE: pulsed field gel electrophoresis; ST: sequence type; SNPs: single nucleotide polymorphisms; WGS: whole genome sequencing.

Declarations
Authors’ contributions
JR assembled the data and drafted the manuscript. GCL and CR helped in MIC determination and
synergy, RCR and SS were responsible for whole genome sequencing and bioinformatics analysis, TG, ASL and VR helped with the draft of manuscript and SFC designed, supervised the study and did analysis, interpretation and drafted the manuscript. All authors have read, contributed and approved the final manuscript.

Funding

Internal funding from the University of São Paulo, Brazil and the National Counsel of Technological and Scientific Development (CNPQ), Brazil supported this study.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval

These experiments were approved by the Ethical Committee of Hospital das Clinicas of University of Sao Paulo and received approval by CONEP (National Ethics Commission), Brazil.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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**Tables**

Table 1. Baseline characteristics of 28 hematopoietic stem cell transplant patients that developed bacteraemia caused by carbapenem-resistant *P. aeruginosa*. 
| Characteristics                                      | Total   |
|-----------------------------------------------------|---------|
|                                                     | n=28 (%)|
| Gender, Female                                      | 18 (64) |
| Age (years)                                         |         |
| Median (range)                                      | 42,4 (16-63) |
| Underlying disease                                  |         |
| Acute leukaemia                                     | 12 (43) |
| Lymphomas                                           | 6 (21)  |
| Multiple myeloma                                    | 5 (18)  |
| Aplasia                                             | 4 (14)  |
| Granulocytic sarcoma                                | 1 (3)   |
| Disease status at transplant                        |         |
| Complete response                                   | 6 (21)  |
| Partial response or active disease                  | 22 (79) |
| Type of HSCT                                        |         |
| Autologous                                          | 9 (32)  |
| Allogeneic                                          | 19 (68) |
| Length hospital stay before infection (days)        |         |
| Median (range)                                      | 19 (4 - 64) |
| Time between HSCT and infection (days)              |         |
| Median (range)                                      | 9,5 (0 - 87) |
| Neutropenia, Yes                                    | 26 (93) |
| Mucositis*, Yes                                     | 17/22 (77) |
| Pitt score                                          |         |
| Mean (SD)                                           | 1,17 ± 2,64 |
| Patients with score ≥ 4                            | 4 (14)  |
| Prior gut colonization * Yes                         | 8/25 (32) |
| Initial antibiotic < 24h                            | 16 (57) |
| Polymicrobial infection*                            | 3 (9)   |
| 7-Day mortality                                     | 19 (68) |
| 14-Day mortality                                    | 20 (71) |

# Data not available for 6 and 3 patients respectively  * Concomitant infection due to Enterococcus faecium, E. coli and Staphylococcus haemolyticus.  HSCT: Hematopoietic stem cell transplant

Table 2. Microbiological characteristics of thirty isolates of carbapenem-resistant P. aeruginosa in bloodstream infection.
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| 5 | 4 | S | 1 | S |   |
| 6 | 4 | S | 1 | S |   |
| 7 | 4 | S | 1 | S | R  |
| 8 | 8 | S | 0 | S |   |
| 9 | 5 | R | 0 | S |   |
| 10| 5 | R | 1 | S |   |
| 11| R | 1 | S | R  |
| 12| R | 1 | S | R  |
| 13| R | 0 | S | R  |
| 14| R | 0 | S |   |
| 15| R | 1 | S | R  |
| 16| R | 0 | S | R  |
| 17| R | 0 | S | R  |
| 18| S | 0 | S | R  |
| 19| R | 1 | S | R  |
| 20| R | 1 | S | R  |
| 21| R | 0 | S | R  |
| 22| S | 0 | S | R  |
| 23| 4 | S | 0 | S | R  |
| 24| R | 0 | S | R  |
| 25| R | 0 | S | R  |
| 26| S | 0 | S | R  |
| 27| R | 4 | I | R  |
| 28| R | 4 | I | R  |
| 29| R | 4 | I | R  |
| 30| R | 4 | I | R  |
MIC: Minimum inhibitory concentration, S: susceptible, R: resistant, I: intermediate, PCR: polymerase chain reaction, COL: colistin, MERO: meropenem, AMK: amikacin, The strains (1,8,10, 13,14,15,19,28) sequenced by illumine were assigned as ST27, and harboured the follow resistance genes: \textit{aacA4} – Aminoglycoside N(6')-acetyltransferase type 1; \textit{aac(6')Ib-cr} – Aminoglycoside 6'-N-acetyltransferase Ib-Cr type; \textit{aadA7} – Streptomycin 3”-adenylyltransferase; \textit{aph(3')-llb} – Aminoglycoside-phosphotransferase; \textit{bla_{OXA-50}} – Oxacillinase; \textit{bla_{OXA-56}} – Oxacillinase; \textit{bla_{PAO}} – Beta-lactamase; \textit{bla_{SPM-1}} – Beta-lactamase; \textit{catB7} – Chloramphenicol acetyltransferase; \textit{cmx} – Chloramphenicol resistance protein; \textit{fosA} – Glutathione transferase; \textit{rmtD} – 16S rRNA methylase; \textit{sul1} – Sulfonamide-resistant dihydropteroate synthase; except strain14 carbapenemase negative), strain 10 rmtD, fosA and sulf1 negative and strain 28 rmtD negative, RG: bacterial re-grow

Table 3. Comparison of clinical and laboratorial features of 25 patients submitted to HSCT with carbapenem-resistant \textit{P. aeruginosa} BSI appropriated treated regarding outcome – São Paulo, Brazil.

| SURVIVED (n=6) | DIED (n=19) | p Value |
|----------------|-------------|---------|
|                | n (%)       | n (%)   |         |
| Age (mean ± SD)| 52.33 ± 11.55 | 41.05 ± 15.27 | 0       |
| Male gender    | 1 (17)       | 7 (37)  | 0       |
| Previous leukaemia | 0          | 9 (47)  | 0       |
| HSCT allogeneic| 1 (17)       | 15 (78) | 0       |
| Neutropenia    | 6 (100)      | 18 (94) | 1       |
| Pitt score (mean ± SD) | 0 ± 0 (n=6) | 1.58 ± 3.15 (n=19) | 0 |
| Previous gut colonization | 3 (50) | 4 (23) | 0 |
| Treatment < 24h| 3 (50)       | 13 (68) | 0       |

\textit{In vitro} synergy

| COL + MERO       | 6 (100) | 9 (47) | 0 |
| COL + AMK        | 2 (33)  | 0      | 0 |
| MERO + AMK       | 3 (50)  | 3 (16) | 0 |
| \textit{Carbapenamase genes by PCR} | |
| \textit{bla_{SPM}} | 4 (66) | 10 (53) | 0 |
| \textit{bla_{SPM}} and \textit{bla_{KPC}} | 0 (0) | 4 (21) | 0 |
| \textit{Virulence genes by PCR} | |
| \textit{lasB}    | 3 (50)  | 17 (89) | 0 |
| \textit{ExoS; phzM and toxA} | 6 (100) | 18 (96) | 1 |
| \textit{Treatment received} | |
| 2 antibiotics (COL plus MERO) | 5 (71) | 10 (56) | 0 |
| 3 antibiotics (COL plus MERO plus AMK) | 2 (29) | 8 (44) | |

HSCT: Hematopoietic stem cell transplant, BSI: bloodstream infection, SD: standard deviation, COL:
Table 4. Resistance and virulence genes by whole genome sequence of main clones of carbapenem-resistant *P. aeruginosa* causing bloodstream infection in HSCT patients.

| Isolate number | Tn   | Resistance genes                                                                 | Quorum-sensing | Biofilm          |
|----------------|------|----------------------------------------------------------------------------------|----------------|------------------|
|                |      |                                                                                  |                |                  |
| 4371           |      | **aacA4, aac(6')Ib-cr, aadA7, aph(3')-Ilb, blaOXA-50, blaOXA-56, **blaSPM-1, catB7, cmx, fosA, rmtD, sul1 | **gacA, gacS, ladS, lasB, bfmR, bfmS, qscR, qteE** |                  |
| 1              | Pos  | aacA4, aac(6')Ib-cr, aadA7, aph(3')-Ilb, blaOXA-50, blaOXA-56, **blaSPM-1**, catB7, cmx, fosA, rmtD, sul1 | **gacA, gacS, ladS, lasB, bfmR, bfmS, qscR, qteE** |                  |
| 8              | Pos  | aacA4, aac(6')Ib-cr, aadA7, aph(3')-Ilb, blaOXA-50, blaOXA-56, **blaSPM-1**, catB7, cmx, fosA, sul1 | **gacS, ladS, lasA, lasB, bfmS, qscR** |                  |
| 10             | Pos  | aacA4, aac(6')Ib-cr, aadA7, aph(3')-Ilb, blaOXA-50, blaOXA-56, **blaSPM-1**, catB7, cmx | **gacA, gacS, ladS, lasB, bfmR, bfmS, qscR, qteE** |                  |
| 13             | Pos  | aacA4, aac(6')Ib-cr, aadA7, aph(3')-Ilb, blaOXA-50, blaOXA-56, **blaSPM-1**, catB7, cmx, fosA, rmtD, sul1 | **gacA, gacS, ladS, lasB, bfmR, bfmS, qscR, qteE** |                  |
| 14             | Pos  | aacA4, aac(6')Ib-cr, aadA7, aph(3')-Ilb, blaOXA-50, blaOXA-56, **blaSPM-1**, catB7, cmx, fosA, rmtD, sul1 | **gacA, gacS, ladS, lasB, bfmR, bfmS, qscR, qteE** |                  |
| 15             | Pos  | aacA4, aac(6')Ib-cr, aadA7, aph(3')-Ilb, blaOXA-50, blaOXA-56, **blaSPM-1**, catB7, cmx, fosA, rmtD, sul1 | **gacA, gacS, ladS, lasB, bfmR, bfmS, qscR, qteE** |                  |
| 19             | Pos  | aacA4, aac(6')Ib-cr, aadA7, aph(3')-Ilb, blaOXA-50, blaOXA-56, **blaSPM-1**, catB7, cmx, fosA, rmtD, sul1 | **gacA, gacS, ladS, lasB, bfmR, bfmS, qscR** |                  |
| 28             | Pos  | aacA4, aac(6')Ib-cr, aadA7, aph(3')-Ilb, blaOXA-50, blaOXA-56, **blaSPM-1**, catB7, cmx, fosA, sul1 | **gacA** |                  |

ST, Sequence Type, Pos, Positive. **aacA4** – Aminoglycoside N(6')-acetyltransferase type 1; **aac(6')Ib-cr** – Aminoglycoside 6′-N-acetyltransferase Ib-Cr type; **aadA7** – Streptomycin 3″-adenylyltransferase; **aph(3')-Ilb** – Aminoglycoside-phosphotransferase; **blaOXA-50** – Oxacillinase; **blaOXA-56** – Oxacillinase; **blaPAO** – Beta-lactamase; **blaSPM-1** – Beta-lactamase; **catB7** – Chloramphenicol acetyltransferase; **cmx** – Chloramphenicol resistance protein; **fosA** – Glutathione transferase; **rmtD** – 16S rRNA methylase; **sul1** – Sulfonamide-resistant dihydropteroate synthase; **gacA** – response regulator; **gacS** – sensor protein; **ladS** – lost adherence sensor; **lasA** – protease, **lasB** – elastase; **bfmR** – response regulator; **bfmS** – sensor kinase; **qscR** – quorum-sensing control repressor; **qteE** – quorum threshold expression.
element; *lecB* - fucose-binding lectin PA-II; *csuD* - type 1 pili usher protein CsuD; *exoT* - exoenzyme T; *exoY* - adenylate cyclase; *exoU* - phospholipase protein; *toxA* - exotoxin A; *phzM* - phenazine-specific methyltransferase

Figures

Figure 1

Dendrogram showing the relatedness of PFGE patterns among thirty isolates of *Pseudomonas aeruginosa* in BSI construct using 0.5 optimization and 1.5 tolerance.
