γ-Glutamyl Spermine Synthetase PauA2 as Potential Target of Antibiotic Development against *Pseudomonas aeruginosa*

Xiangyu Yao\(^a\), Congran Li\(^b\), Jianmei Zhang\(^b\), and Chung-Dar Lu\(^a\textsuperscript{c}\textsuperscript{+}\textsuperscript{*}

Department of Biology, Georgia State University, Atlanta, Georgia, USA\(^a\), Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, PR China\(^b\), and Department of Medical Laboratory Sciences and Biotechnology, China Medical University, Taichung, Taiwan\(^c\)

Running title – glutamyl spermine synthetase as drug target

*Corresponding author. Mailing address: Department of Biology, Georgia State University, 100 Piedmont Avenue, Atlanta, Georgia 30303. Phone: 404-413-5395. Fax: 404-413-5301. Email: biocdl@gsu.edu.

\(^+\)X. Yao and C. Li contribute equally to this work.
Polyamines are absolute requirements for cell growth. When in excess, P. aeruginosa possesses six γ-glutamylpolyamine synthetases (GPSs) encoded by the pauA1-pauA7 genes to initiate polyamine catabolism. Recently, the pauA2 mutant was reported to lose the capability to grow on spermine (Spm) and spermidine (Spd) as sole carbon and nitrogen sources. Although this mutant grew normally in the defined minimal medium and the LB broth, growth was completely abolished by the addition of Spm or Spd. These two compounds exert a bactericidal effect (Spm > Spd) on the mutants as demonstrated by MIC measurements (over 500-fold reduction) and time-killing curves. Spm toxicity in the pauA2 mutant was attenuated when the major uptake system was further deleted from the strain, suggesting cytoplasmic targets of toxicity. In addition, the synergistic effect of Spm and carbenicillin in the wild type strain PAO1 was diminished in mutants without functional PauA2. Furthermore, Spm MIC was reduced by 8-fold when deleting the Spm uptake system from the wild type strain, suggesting a second target of Spm toxicity in the periplasm. Experiments were also conducted to test the hypothesis that native Spm and Spd in human serum may be sufficient to kill the pauA2 mutant. Growth of the mutant was completely inhibited by 40% (v/v) human serum while the parental strain required 80%. Colony counts indicated that the mutant but not the parent was in fact killed by human plasma. In addition, carbenicillin MIC against the mutant was reduced by 16-fold in the presence of 20% human serum while that of the parental strain remained unchanged. Taking PauA2 as template, sequence comparison indicates that putative PauA2 homologues are widespread in a variety of Gram-negative bacteria. In summary, this study reveals the importance of GPS in alleviation of
polyamine toxicity when in excess, and provides strong support to the feasibility of GPS as a molecular target for new antibiotic development.

INTRODUCTION

Spermine (Spm) along with spermidine (Spd) and putrescine (Put) are the three most common polyamines in human and other eukaryotic cells (3). Because of the positive-charged nature, polyamines are believed to serve as ubiquitous structural components in cells through interactions with RNA and DNA macromolecules. Considerable attention has been paid to polyamine biosynthesis as a target for anti-tumor therapy because an elevated level of intracellular polyamine pools is a unique character of tumor cells (21, 25). Spermine also participates in regulation of innate immunity. The tissue level of spermine is significantly increased at the inflammatory sites of infection. Released from dying cells, spermine can restrain macrophage activation in the anti-inflammatory process by inhibiting pro-inflammatory gene expression (24, 30).

Bacteria also need polyamines for growth. Unlike eukaryotes, most bacteria don’t make spermine. It has been reported that mutant strains of *E. coli* and *P. aeruginosa* defective in polyamine biosynthesis exhibited a retarded growth phenotype (2, 17). Based on the studies in polyamine-requiring mutant strains of *E. coli*, the concept of “polyamine modulon” was proposed by Igarashi and Kashiwagi (7), referring to a group of genes whose expression requires...
intracellular polyamines for translational initiation. In vitro, exogenous polyamines have been reported to participate in many other aspects of bacterial physiology including inhibition of swarming ability and chemotaxis (4), resistance to nitrosative stress (1), activation of biofilm formation (9), anti-mutagens activity (19-20), and SOS induction of RecA and UvrA (11). Exogenous polyamines and the cognate uptake systems also play roles in bacterial virulence (31) and drug resistance. We have reported several significant effects of exogenous polyamines on antibiotics (13-14). In particular, spermine increased the susceptibility of both Gram-negative *P. aeruginosa* and *E. coli* and Gram-positive methicillin-resistance *Staphylococcus aureus* (MRSA) to β-lactam antibiotics. However, molecular mechanisms for many of these multifaceted polyamine effects remain unknown.

Although polyamines are required for cell growth, these compounds could be very toxic if in excess. Depending on the pH value in the environments, polyamines can be highly positive-charged molecules and potent nucleophiles. Through charge interactions these compounds can cause DNA condensation in vitro (18), and as nucleophiles they are able to form covalent adducts with β-lactams (29) and potentially with other signal compounds or protein targets. To avoid these potential lethal effects, the cells need to maintain polyamine homeostasis through coordinated regulation on biosynthesis, catabolism, and uptake.

Polyamine catabolism can potentially attenuate the toxicity through modification on the amine groups, and ultimately degrade the compounds. As shown in Fig. 1, polyamines are subjected to acetylation by spermidine/spermine acetyltransferase (SSAT) or γ-glutamylation by glutamylpolyamine synthetase (GPS) to initiate degradation. Human cells take the SSAT route to
degrade and recycle spermidine and spermine, or pump acetylated products out of the cells. In bacteria, the function of SSAT has been reported in *E. coli* (5, 15), *B. subtilis* (26), and a specific strain lineage of *S. aureus* (8). *P. aeruginosa* and related bacteria do not seem to have an apparent SSAT homologue based on sequence comparison. Instead, our recent report (28) supports the presence of seven GPSs encoded by the *pauA1*-pauA7 genes in polyamine catabolism. While some of these GPSs share common substrates, Spm and Spd catabolism requires absolutely the presence of a specific GPS – PauA2.

The PauA2 homologues are widely spread in Gram-negative bacteria. These enzymes were initially annotated as glutamine synthetase homologues based on sequence similarities. The first reported case of this family is PuuA of *E. coli*, which preferentially takes Put and other diamines as substrate (12). Through the glutamylation pathway, one primary amine of putrescine, and presumably polyamines in general, is converted into carboxylic acid with the release of ammonium and recycle of glutamate. While *E. coli* was reported to possess one single set of genes for this pathway, the presence of redundant enzymes in pseudomonad expands the capacity of these bacteria to utilize a variety of polyamines as carbon and nitrogen sources.

In this study we further demonstrated the importance of PauA2 and Spm uptake against Spm toxicity and in Spm homeostasis of *P. aeruginosa*. Experiments were also conducted to test the hypothesis that mutants of *P. aeruginosa* without a functional PauA2 might be vulnerable to the intrinsic polyamine contents in human serum.
Strains and growth conditions. Bacterial strains used in this study are *P. aeruginosa* PAO1 and mutants derived from PAO1. Construction and characterization of strain M1A2 (ΔpauA2), M7A (ΔpauA1-pauA7), and PAO5011 (spuF) have been reported (16, 28). To introduce the *spuF* allele into M1A2 and M7A, *E. coli* SM10 carrying the corresponding plasmid for allele exchange biparental conjugation was mated with the recipient strains following the protocol as described previously (16). The bacteria were routinely cultured in the Luria-Bertani (LB) enriched medium at 37 °C.

MIC determination. Minimal inhibition concentrations (MICs) of antibiotics or spermine were determined by broth micro-dilution method in 96-well titer plates according to the CLSI guideline. Serial two-fold dilutions of tested compounds were prepared in the LB broth. When required, the LB medium was buffered with 20mM Tris-HCl of indicated pH. Fresh overnight cultures of each bacterial strain were diluted and inoculated with approximate 5 x10^5 CFU/well. The plates were properly wrapped and incubated without shaking at 37 ºC for 16-18 h. MIC was defined as the lowest concentration of the agent that inhibited the growth of the bacteria as detected by the unaided eyes. The MICs were determined in triplicate.

Checkerboard testing. Checkerboard tests were performed in triplicate for the combination of carbenicillin plus spermine in the LB broth (pH 8.0). The concentrations of antimicrobials ranged from approximately 4xMIC to seven serial twofold dilutions below this amount. Each plate also contained a row and column in which a serial dilution of each agent was present alone.
Bacteria with an inoculum of approximately 5 × 10^5 CFU were then added to each well, and the plates were incubated at 37°C for 18h. Synergy was measured by determination of the FIC, which is the ratio of the MIC of a drug in combination and MIC of the drug alone. For two interacting compounds A and B, the sum of the FICs (ΣFIC = FIC_A + FIC_B) indicates the extent of the interaction. When ΣFIC is ≤0.5, there is a synergistic effect. A ΣFIC of 0.5 to 4.0 is defined as indifference, and antagonism is defined as a ΣFIC of ≥4.0.

**Time-killing assay.** In vitro killing curve studies were performed as described previously. The bacterial culture was diluted in LB broth to an OD_600 reading of 0.15, and an aliquot of the diluted cells (final inoculum of approximately 1.5 x 10^5 CFU) was inoculated into the LB broth (pH 8.0) in the presence or absence of the indicated polyamines (10mM spm or 10mM spd). The culture was incubated at 37°C with shaking at 180rpm. After 0, 1, 2, 4, and 6h, samples were withdrawn and plated onto LB agar plates to obtain viable colony counts (serial dilutions were prepared when necessary). Bacterial colonies on the plates were counted after 18 to 24 h of incubation at 37°C. Experiments were performed in duplicates.

**Measurements of human plasma and serum effects on bacterial growth.** Pooled human plasma was obtained by anticoagulation with heparin and sterilized by filtration before use. Growth media containing indicated percentage of plasma were prepared by mixing with appropriate proportions of cation-adjusted Mueller-Hinton broth (CAMH). The mixtures were aliquoted to 96 well plates, and inoculation of the three strains was performed as in MIC determination. The plates were properly wrapped and incubated at 35°C for 16-18 h. The growth of the cells in each well was recorded and viable cells were counted for wells containing 0, 10,
50, 80, 90 and 100% human plasma. The effect of human serum on bacterial growth was also tested in a similar way as described for plasma. The effect of human serum on the antibacterial activity of carbenicillin against strains of *P. aeruginosa* was tested in serum concentrations of 0% to 50%.

**RESULTS AND DISCUSSION**

Enhanced spermidine and spermine toxicity in the mutant strains of *P. aeruginosa* devoid of PauA2. In *P. aeruginosa* PAO1 there are seven potential GPSs (PauA1-A7), which were proposed to participate in polyamine catabolism via the γ-glutamylation pathway (28). Growth of PAO1 on different polyamines requires a distinct set of PauA combinations, e.g. PauA1A2A4 for Put, PauA1A4A5 for cadaverine, and PauA2 for Spd and Spm (28). The M7A mutant devoid of seven PauA enzymes cannot grow on all tested polyamines as sole source of carbon and nitrogen. However, the “no-growth” phenotype of the M7A or PauA2 mutant on Spd or Spm was not simply due to the lack of catabolism. We found that these mutants grew normally in the LB medium, but growth was arrested in LB supplemented with 10 mM of Spm or Spd (data not shown). In comparison, addition of Put did not affect growth of M7A. The same pattern of growth phenotypes was also observed in the glutamate minimal medium with or without polyamine supplements. These results indicated that growth of these mutants without a functional PauA2 is subjected to inhibition by exogenous Spm and Spd.
To further differentiate the adverse effect of polyamines, MICs of these compounds were measured by the liquid micro-dilution method. As shown in Table 1, MICs of Spd and Spm reduce from 160 mM and 80 mM in the parental strain PAO1 to 5 mM and 0.3 mM, respectively, in the pauA2 and M7A mutants. In contrast, Put MIC remains unchanged (>160 mM) even in the M7A mutant. These results support the function of PauA2 in detoxification of Spm and Spd through glutamylation, and suggest the presence of a Spm-sensitive intracellular target of unknown nature.

The bactericidal effect of Spd and Spm. The inhibitory effect of Spd and Spm on growth was also assessed by time-killing assays. As shown in Fig. 2, growth of the pauA2 mutant in the LB broth continues for at least 6 hours. In contrast, Spd and Spm at 10 mM exert a killing effect on the pauA2 mutant; no apparent viability of the inoculated cell population can be detected after 6-hour exposure. The killing kinetics also revealed that Spm exerts a much stronger effect than Spd does. These results indicate that Spd and Spm, if not modified by PauA2-dependent glutamylation, have a potent bactericidal effect on P. aeruginosa.

Endogenous Spm and Spd were known to interact with the bacterial ribosome (22-23). Under normal physiological conditions, one proposed function of Spm and Spd for cell viability is to ensure structural and functional integrity of the ribosome by binding to a specific region on the 23S rRNA of E. coli (27). In addition, Spm and Spd can also bind to tRNAs and result in acceleration of codon recognition on the ribosome (6). However, the character of positive charges carried by polyamines has long been considered as potential adverse effects if these essential compounds were accumulated to high concentrations inside the cells. To demonstrate
the potential toxic effect of polyamines by their charge interactions with nucleic acid polymers, we tested the DNA-binding activity of Spm, Spd, and Put \textit{in vitro}. When bound to DNA, polyamines are expected to reduction of overall net negative charges of DNA-polyamine complexes, and hence the mobility of these complexes on the native PAGE. Formation of these complexes would be reflected on decreased amounts of free-form DNA; no distinct retarded complex can be observed by this assay. As shown in Fig. 3, the intrinsic DNA-binding affinity of Spm is about 10-fold higher than that of Spd, and no apparent binding by Put can be detected even at the conc. of 5 mM. This trend of DNA affinity (Spm > Spd > Put) is consistent to that of toxicity by MIC measurements (Table 1).

**Effects of uptake on Spm toxicity.** Since \textit{P. aeruginosa} does not make Spm through \textit{de novo} synthesis, homeostasis of Spm would be mainly controlled by uptake and catabolism. As described above, PauA2 plays a pivotal role in Spm catabolism. Among potential uptake systems for polyamines in \textit{P. aeruginosa} genome annotations (www.pseudomonas.com), we have reported that the \textit{spuEFGH} operon encodes components of an ABC transport system for Spd in \textit{P. aeruginosa} (16). Uptake of exogenous Spd was greatly diminished without a functional SpuEFGH transporter, and the mutant of which cannot grow on Spd as the sole carbon source. Based on the structural similarities, we proposed that the SpuEFGH transporter also serves for Spm uptake. To analyze the potential effects of uptake and catabolism in Spm homeostasis, and hence Spm-dependent toxicity and synergy, a \textit{spuF} knockout lesion was introduced in the wild type strain PAO1 and the \textit{pauA2} mutant, and Spm MICs of these strains were shown in Table 1.
If the spuF knockout lesion indeed blocked Spm uptake, one would expect an increased Spm MIC in the corresponding mutants regardless the presence or absence of a functional PauA2.

Surprisingly, in the presence of a functional PauA2, Spm MIC of the spuF mutant was 8-fold lower than that of PAO1 (10 mM vs. 80 mM). However, in the absence of PauA2, the spuF pauA2 mutant indeed exhibited a Spm MIC 4-fold higher than that of the pauA2 mutant (1.2 mM vs. 0.3 mM). Information revealed by these somewhat contradictory results led to the following working model for the maintenance of Spm homeostasis in P. aeruginosa.

As shown in Fig. 4, two new molecular elements were proposed in this “two-mode action” model – (i) a second Spm transport system with uptake efficiency much lower than that of SpuEFGH; and (ii) a Spm-dependent cytotoxic target in the periplasm. In the wild type strain PAO1, exogenous Spm can pass the outer membrane barrier through unknown porins to reach the periplasm, and subsequently taken into the cytoplasm by specific transporters. Intracellular Spm will then be modified by PauA2-dependent γ-glutamylation and subjected to further degradation to support cell growth as carbon and nitrogen sources. In the spuF mutant, exogenous Spm accumulates in the periplasm to a level significantly higher than that in the wild type strain PAO1 because the absence of a transporter of high-efficiency. Periplasmic Spm at a relatively high concentration may interact with and damage a molecular target essential for cell growth, perhaps through its strong nucleophilic property. Significant reduction of Spm MIC in the spuF mutant in comparison to PAO1 may be explained by adverse effects of Spm when accumulated in the periplasm of this mutant. In the pauA2 mutant, a strong Spm influx into the cytoplasm but no attenuation by γ-glutamylation certainly makes this mutant more vulnerable to the Spm-dependent cytotoxic effect on an intracellular target. Without PauA2 and SpuF, the
residual level of Spm uptake through the second transport system remains sufficient to cause
accumulation of intracellular Spm to a level that leads to cell death. One likely candidate for the
second transport system is encoded by PA3607-PA3610, which were annotated as potABCD
based on sequence similarities to the *E. coli* counterparts (10) that encode components of a Spd
transport system.

Dasu *et al* have reported a periplasmic spd/spm dehydrogenase (3). Although this enzyme alone
may not have any significant contribution to polyamine catabolism since its expression level was
constitutively low and not inducible by polyamines, it may provide some degree of protection
against spm/spd toxicity in the *spuF* mutant.

**No synergy by Spm and β-lactam antibiotics in the pauA2 mutant.** Exogenous polyamines
exert multiple effects on cell physiology, very likely mediated by interactions with different
molecular targets. One particular effect of exogenous Spm that we have been interested in is the
synergistic effect of Spm on β-lactam antibiotics. As proposed in the current working model,
there are two Spm-dependent targets, one in the cytoplasm and another in the periplasm. To
further understand the potential role of these two targets on synergy, we conducted checkerboard
analysis in PAO1 and its mutants of *pauA2* and/or *spuF*. As shown in Fig. 5, the calculated FIC
index clearly indicated strong synergy with Spm and carbenicillin against the wild type PAO1. In
the *pauA2* mutant, only low concentrations of Spm can be applied because this mutant is much
more sensitive to Spm than PAO1, and no synergy can be detected in this mutant. Without
PauA2-dependent modification, exogenous Spm in low concentrations was sufficient to cause
significant accumulation of free-form Spm inside this mutant through active uptake and resulted
in cell death. The *pauA2 spuF* mutant also exhibited no synergy as it can still uptake and
accumulate Spm through the second transport system as described above (Fig. 4). These results indicated that the intracellular Spm-sensitive target of unknown nature may not be required for synergy. Contrarily, synergy of carbenicillin and Spm persisted in the *spuF* mutant, of which growth inhibition by exogenous Spm was proposed to act on the second target in the periplasm.

One possibility was that this Spm-dependent second target was related to cell wall synthesis. This hypothesis was consistent to our recent finding that in MRSA, a mutation in *pbpB* encoding the penicillin-binding protein 2 for cell wall synthesis can abolish the synergistic effect of exogenous Spm and β-lactams (29).

The *pauA2* mutants are more susceptible to human serum and plasma. Human cells, but not *P. aeruginosa* or many other bacteria, are able to synthesize Spm. Demand for Spm in cancer cells was found stronger than that in normal tissues, and Spm released from damaged tissues was reported to regulate innate immunity. Therefore, we proposed that circulating Spm in human body may be sufficient to post adverse effects on bacteria of no protective mechanisms. To test this hypothesis, survival rates of the *pauA2* mutants and the parental strain PAO1 after exposure to human plasma were measured and compared. The results demonstrated that viable cell counts of *pauA2* and PM7 were over 200 fold lower than the corresponding values of PAO1 when the concentrations of human plasma was at or over 90% (data not shown).

It was noted that heparin, a highly negatively charged compound, was routinely used as anticoagulant in preparation of plasma from pooled blood samples. Since Spm is a positive-charged molecule at the physiological pH of human blood, it is conceivable that interactions of heparin and Spm by charge can reduce the working concentration of free Spm in the plasma.
Therefore, pooled serum was prepared in the absence of heparin to test its effect on bacterial
growth. As shown in Table 2, growth of PAO1 was more sensitive to the presence of serum than
plasma; serum in 80% was sufficient to inhibit growth while it required plasma in 100%. In
comparison, the pauA2 and the M7A mutants were more susceptible than PAO1 to growth
inhibition by plasma (in 80%) and even more so by serum (in 40%).

The potential effect of β-lactam antibiotics in the presence of serum was also analyzed by MIC
measurements. As shown in Table 3, carbenicillin MIC of PAO1 was reduced by 4-fold in the
presence of 20-50% serum (64 vs. 16 ug/ml), while that of the pauA2 mutant was decreased 64
fold in the presence of 20% serum and was below 0.1 ng/ml when 30% serum was added in the
growth medium. These results were surprising as the synergistic effect of Spm and carbenicillin
was lost in the pauA2 mutant (Table 1) when tested in the LB broth. Regardless, it is conceivable
that PauA2 plays important roles in supporting viability of P. aeruginosa in human serum.
Molecular mechanisms of these PauA2-associated processes warrant further investigation.

In summary, spermine has multiple targets in P. aeruginosa. Our previous report indicated that
they can block the outer membrane porin D and hence induce resistance to carbapenem (13), and
that they can trigger the PhoPQ two-component system to modify lipopolysaccharides and result
in resistance to polymyxin B (14). Through genetic studies, here we provide further evidence to
support the presence and possible locations of two more targets. We demonstrated that the
 glutamyl polyamine synthetase PauA2 plays a major role in protection of P. aeruginosa against
the bactericidal effect of spermine. The toxic effects of spermine may have two targets, one
inside and the other outside of the cells. While the intracellular target is more vulnerable without
protection by PauA2, the synergistic effect of spermine and β-lactam antibiotics may come from
the target in the periplasm. Although the pauA2 mutants grew normally in the rich or minimal
medium, they exhibited significant survival disadvantage as well as a pronounced synergistic
effect with carbenicillin by the presence of human serum or plasma. These results support our
hypothesis that circulating spermine in human bodies may potentially provide another layer of
innate immunity against bacterial pathogens when the intrinsic spermine-modifying enzyme was
inactivated. PauA and its homologues can be promising molecular targets for the development of
a new type of antibiotics against *P. aeruginosa* and related bacteria of multiple drug resistance.

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Yao.
REFERENCES


FIGURE LEGENDS

Figure 1. Schematic presentation of common polyamines and two polyamine modification reactions. GPS, glutamylpolyamine synthetase; SSAT, spermine/spermidine acetyltransferase; Glu, glutamate.

Figure 2. Time-killing curves for ΔpauA2. Time-killing curves were performed as described in Materials and Methods in the LB broth alone (none, filled circle), with 10 mM spermine (Spm, filled square), or with 10mM spermidine (Spd, filled triangle). Also shown are error bars for each data point, representing duplicate experiments.

Figure 3. Binding of polyamines to DNA. Equal amounts of a DNA fragment were mixed without or with the indicated conc. of polyamines before electrophoresis. Interactions of polyamine and DNA results in the reduced intensity of DNA fragments.

Figure 4. Summary of the “two-mode action” model of polyamines in P. aeruginosa. The tetra-amine spermine was used as representative. PBPs, penicillin-binding proteins in cell wall synthesis.

Figure 5. Isobolograms of spermine and carbenicillin FIC values against P. aeruginosa. Determination of FICs by the checkerboard assays was described in Materials and Methods. Four strains of P. aeruginosa were used in this study as marked by different labels, and the calculated average sum of the FICs in each strain was displayed in parenthesis.
polyamines

- Diaminopropane
- Putrescine
- Cadaverine
- Spermine
- Spermidine
- Norspermine

\[ \text{Glu + ATP} \rightarrow \text{Glu-ATP} \rightarrow \text{UPS} \]
\[ \text{ADP + Pi} \rightarrow \text{Acetyl-CoA} \rightarrow \text{SSAT} \]
\[ \gamma\text{-Glutamyl polyamine} \rightarrow \text{Acetyl polyamine} \]
Table 1. MICs of polyamines in *P. aeruginosa*

<table>
<thead>
<tr>
<th>Strain (genotype)</th>
<th>MICs (mM) of polyamines</th>
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<tbody>
<tr>
<td></td>
<td>Put</td>
</tr>
<tr>
<td>PAO1 (w.t.)</td>
<td>&gt;160</td>
</tr>
<tr>
<td>M1A2 (ΔpauA2)</td>
<td>&gt;160</td>
</tr>
<tr>
<td>M7A (ΔpauA1-ΔpauA7)</td>
<td>&gt;160</td>
</tr>
<tr>
<td>PAO5011 (ΔspuF)</td>
<td>&gt;160</td>
</tr>
<tr>
<td>M1A2F (ΔpauA2ΔspuF)</td>
<td>&gt;160</td>
</tr>
</tbody>
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*MICs were determined in LB broth with pH adjusted to 8. Put, Putrescine; Spm, spermine; Spd, spermidine.*
Table 2. Effects of human plasma and serum on growth of *P. aeruginosa*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth of the Strains in Plasma or Serum Concentration of (%)</th>
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<tbody>
<tr>
<td></td>
<td>100%</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
</tr>
<tr>
<td>PAO1</td>
<td>-</td>
</tr>
<tr>
<td>M1A2</td>
<td>-</td>
</tr>
<tr>
<td>M7A</td>
<td>-</td>
</tr>
<tr>
<td><strong>Serum</strong></td>
<td></td>
</tr>
<tr>
<td>PAO1</td>
<td>-</td>
</tr>
<tr>
<td>M1A2</td>
<td>-</td>
</tr>
<tr>
<td>M7A</td>
<td>-</td>
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</tbody>
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Table 3. Effect of human serum on antibacterial activity of carbenicillin against *P. aeruginosa*

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC of Carbenicillin (µg/ml) in Serum Concentration of (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>50%</td>
</tr>
<tr>
<td>PAO1</td>
<td>16</td>
</tr>
<tr>
<td>M1A2</td>
<td>≤0.0001</td>
</tr>
<tr>
<td>M7A</td>
<td>≤0.0001</td>
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