remarkable degree of synergism against *Rhizopus* isolates. Further *in vivo* studies are warranted to explore the potential role of these combinations for the treatment of zygomycosis.

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**References**


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**Piperacillin/tazobactam-heteroresistant *Pseudomonas aeruginosa* from urinary infection, successfully treated by piperacillin/tazobactam**

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Keywords: population analysis, time–kill, heteroresistance, persisters, β-lactams, heterogeneity

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Sir,

Pseudomonal infections have limited therapeutic options due to the intrinsic resistance of the microorganism and its ability to integrate further resistance mechanisms. Although antipseudomonal β-lactams such as carbapenems and piperacillin/tazobactam still remain clinically useful, carbapenem-heteroresistant mutants of *Pseudomonas aeruginosa* have recently been described. We wish to further report the occurrence of an isolate showing heteroresistance to piperacillin/tazobactam.

*P. aeruginosa* strain 7171 (PA7171) was isolated in November 2006 from a urinary tract infection sample of a 38-year-old male who was hospitalized for 7 days at the University Hospital of Larissa. Species identification and initial antibiotic susceptibility testing were performed by the Vitek 2 automated system (bioMérieux, Marcy l’Étoile, France), according to the manufacturer’s instructions. Susceptibility to several antipseudomonal drugs was also determined by disc diffusion, agar dilution and Etest (AB Biodisk, Solna, Sweden) methods.

*P. aeruginosa* ATCC 27853 was used as a control in all susceptibility testing assays. By the automated system, PA7171 was reported as non-susceptible to amikacin, aztreonam, cefepime, ciprofloxacin and meropenem and susceptible to ceftazidime, piperacillin/tazobactam, imipenem and colistin. By agar dilution, the isolate was non-susceptible to amikacin (MIC > 256 mg/L), cefepime (64 mg/L), ceftazidime (32 mg/L), ciprofloxacin (>256 mg/L), meropenem (32 mg/L) and aztreonam (16 mg/L) and susceptible to imipenem (2 mg/L) and piperacillin/tazobactam (16 mg/L). According to the agar dilution MICs, the isolate was categorized as susceptible to piperacillin/tazobactam by using both CLSI and BSAC breakpoints. However, the isolate exhibited distinct colonies within the inhibition halo around the piperacillin/tazobactam disc (Figure 1a) and Etest strip, implying the presence of heteroresistant subpopulations.

In order to investigate the possibility of heteroresistance to piperacillin/tazobactam, population analysis was undertaken as previously described. The analysis was performed in triplicate, and the mean values of resistant cfu were estimated and plotted on a semi-logarithmic graph. Population analysis of PA7171 was compared with that of ATCC 27853. It was shown that PA7171 grew in the presence of piperacillin/tazobactam up to a concentration of 128 mg/L with a frequency of ~10−7 (Figure 1b). Time–kill kinetics utilizing piperacillin/tazobactam were examined in PA7171 and *P. aeruginosa* ATCC 27853. In this assay, PA7171 exhibited a bactericidal curve similar to that of ATCC 27853 (Figure 1c). Particularly, cell populations gradually decreased and were eliminated within 24 h, whereas no cell regrowth was detected.

When the heteroresistant colonies grown in the highest drug concentration were subcultured weekly in antibiotic-free medium and re-tested by disc diffusion, agar dilution and Etest, the piperacillin/tazobactam agar dilution MIC was the same as that of the native population (16 mg/L). Again, a subpopulation of heteroresistant cells grew within the zone of inhibition around the antibiotic disc or Etest strip, similar to the situation previously described for carbapenem-heteroresistant *Acinetobacter baumannii* clinical isolates. It should be noted that after the subcultures in antibiotic-free medium, considerably less colonies were grown within the inhibition halo.
The patient had not received any treatment prior to the urine sampling. He was empirically treated with 5.2 g of intravenous piperacillin/tazobactam (Tazocin®, Wyeth Pharmaceuticals) every 8 h. Due to a good clinical response, the treatment was not changed after the notification of the heteroresistance to piperacillin/tazobactam. No growth was noticed in repeated urine cultures 1 week after the end of treatment with piperacillin/tazobactam and 1 month after his hospital discharge.

We have previously described heteroresistance to carbapenems in *P. aeruginosa* and *A. baumannii*. In the present study, we analyse the heterogeneous mode of growth of *P. aeruginosa* in piperacillin/tazobactam. As this heteroresistant phenotype has not been reported previously in pseudomonads, it is not known what percentage of *P. aeruginosa* isolates are heteroresistant to the drug and what clinical impact this heteroresistance may have. Also, whether the use of piperacillin/tazobactam may gradually lead to the selection of mutant subpopulations that might subsequently compromise treatment still remains unknown.

In *P. aeruginosa*, a heterogeneous mode of resistance to β-lactams has been shown to emerge readily in hypermutable strains. The heteroresistant phenotype of our isolate resembles persister cells rather than mutants, due to the fact that the heteroresistant subpopulations return to the native phenotype when re-tested and that might partially explain the successful treatment with piperacillin/tazobactam. The fact that the drug achieves high urine concentrations may also have contributed to the favourable outcome. A similar strategy of bacteria to develop persister cells under antibiotic pressure has also been observed in *A. baumannii*. Should antimicrobials against which bacteria produce persisters still remain effective, as shown for piperacillin/tazobactam in *P. aeruginosa*, it is important when it comes to the treatment of multidrug-resistant pseudomonal infections. Larger studies are necessary to determine the frequency of infections due to piperacillin/tazobactam-heteroresistant isolates and their therapeutic consequences.

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**Transparency declarations**

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**References**


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**High isolation rate of *Staphylococcus aureus* from surgical site infections in an Indian hospital**


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