Journal of Microbiology, Immunology and Infection 56 (2023) 344—350

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Original Article

Development of a combination antibiogram for empirical treatments of *Pseudomonas aeruginosa* at a university-affiliated teaching hospital

Ming-Ying Ai [a,b,c], Huai-En Lu [a], Wan-Yu Lee [a], Hsin-Yi Liu [d], Han-Chuan Chuang [d], Bi-Li Chen [a,b], Er-Ying Wang [a,b], Li-Hsin Tsao [e], Yuarn-Jang Lee [d,f,g,*]

[a] *Department of Pharmacy, Taipei Medical University Hospital, Taipei, Taiwan*
[b] *School of Pharmacy, College of Pharmacy, Taipei Medical University, Taipei, Taiwan*
[c] *Department of Pharmacy, Far Eastern Memorial Hospital, New Taipei City, Taiwan*
[d] *Division of Infectious Diseases, Department of Internal Medicine, Taipei Medical University Hospital, Taipei, Taiwan*
[e] *Division of Infectious Diseases, Department of Internal Medicine, Lienchiang County Hospital, Matsu, Taiwan*
[f] *Division of Infectious Diseases, Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taiwan*
[g] *Division of Infectious Diseases, Department of Internal Medicine, Shuang Ho Hospital, Taipei Medical University, New Taipei City, Taiwan*

Received 23 December 2021; received in revised form 16 August 2022; accepted 19 August 2022
Available online 8 September 2022

KEYWORDS
Pseudomonas aeruginosa (PA);
Combination antibiogram;
Empirical combination regimen;
Expand coverage rate;

Abstract *Introduction:* The significantly higher mortality rate in the critical illness patients with *Pseudomonas aeruginosa* (PA) infection is linked to inappropriate selecting of empirical treatment. Traditional local antibiogram provides clinicians the resistant rate of a single antimicrobial agent to the pathogen in the specific setting. The information is valuable to the clinicians in selecting suitable empirical antibiotic therapy. However, traditional local antibiogram can only provide information for single agent empirical antibiotic not combination regimens. The combination antibiogram should be developed to facilitate the selection of appropriate antibiotics to broader the coverage rate of resistant PA.
Methods: The susceptibility to the β-lactam antibiotics (piperacillin/tazobactam (PTZ), ceftazidime, cefepime, imipenem, or meropenem) or to those administered in combination with an aminoglycoside (gentamicin or amikacin) or fluoroquinolone (ciprofloxacin or levofloxacin) was

* Corresponding author. Division of Infectious Diseases, Department of Internal Medicine, Shuang Ho Hospital, Taipei Medical University, No. 291, Zhongzheng Rd., Zhonghe Dist., New Taipei City 235041, Taiwan.
E-mail address: 22001@s.tmu.edu.tw (Y.-J. Lee).

https://doi.org/10.1016/j.jmii.2022.08.012

Local resistant patterns

calculated. The chi-square test was used to compare the differences of combination coverage rates between non-ICU and ICU isolates.
Results: 880 PA isolates were isolated during study period. The susceptibility of single agents ranged from 83.1% to 89.7%. The combination regimens containing amikacin provide the highest cover rate (98.9%—99.1%) and those containing levofloxacin provide less coverage rate (92.3%—93.9%). The susceptibility to five β-lactam single agents in ICU isolates significantly lower than non-ICU isolates. The non-ICU isolates exhibited significantly higher susceptibility to the PTZ—gentamicin ($p = 0.002$) and ceftazidime—gentamicin ($p = 0.025$) than ICU isolates.
Conclusion: Our results support the use of aminoglycosides instead of fluoroquinolones as additive agents in empirical combination treatments for patients with critical infections caused by PA.

Introduction

Pseudomonas aeruginosa (PA) is one of the most common life-threatening bacteria that cause nosocomial infections, which are a major cause of morbidity and mortality, especially in the immunocompromised hosts.[1] The high mortality rate of PA bacteremia is significantly associated with cardiovascular disease and certain phenotypes of carbapenem-resistant *P. aeruginosa* (CRPA) or multidrug resistant PA (MDR-PA).[2] A meta-analysis reported that the decision-making process for selecting an appropriate empirical therapy regimen is key to reduce sepsis-associated mortality.[3]

Piperacillin/tazobactam (PTZ), cefepime, ceftazidime, imipenem, and meropenem are widely used to treat severe PA infections in the intensive care units (ICUs). The excellent bactericidal activity of these β-lactam antibiotics makes them the most crucial empirical option to treat PA. However, the resistance of PA to β-lactam antibiotics has been increasing lately, and the development of new β-lactam antibiotics has not kept pace with this trend.[4] Previous studies have demonstrated that using combination therapy with different classes of drugs successfully reduces mortality among patients with severe MDR-PA infections.[5,6] While there is no international consensus on the definition of multi-drug resistance, most published studies considered that multi-drug resistant as to resistant to at least one drug from three different antibiotic classes, mainly aminoglycosides, antipseudomonal penicillins, cephalosporins, carbapenem and fluoroquinolones. Although the routine use of dual antibiotics remains controversial, one published systematic review and meta-analysis study concluded that combination therapy may reduce mortality for patient who infected with carbapenemase-producing, MDR, or extensively drug-resistant (XDR) Gram-negative bacteria.[7,8]

The selection of antimicrobial agents for use in PA empirical therapy should always consider local resistance patterns. Traditional local antibiograms usually help prescribers to select the optimal empirical single-antibiotic treatment[9]; however, they do not reflect the real antimicrobial resistance conditions when two antimicrobial agents are combined. Combination antibiograms should be developed to help physicians in selecting appropriate combinations of antimicrobial agents to expand the coverage of resistant PA. The cancer patients infected with multidrug resistant Gram-negative bacilli (MDR-GNB) received adequate empirical antibiotic therapy presented with better outcomes and lower overall mortality.[10,11] Kang et al. study showed that there was a trend toward higher mortality in patient with PA bacteremia when effective antimicrobial therapy was delayed.[12] One study had mention that empirical therapy was only adequate in 67% episode of PA infection. However, adding amikacin to a β-lactam antibiotic increased adequacy to 96%.[13] Another studied published in 2021 also indicated that the addition of aminoglycoside or fluroquinolone to cefepime and meropenem could expand empirical coverage rate to above 95%.[14] The aimed of our study is to develop the local combined antibiogram to help clinicians choosing optimal empirical combination regimen for PA infections.

Methods

Settings and population

A retrospective study was conducted at Taipei Medical University Hospital in Taipei, Taiwan from July 1st, 2018 to June 30th, 2019. The number of beds at the hospital was 743 in 2017. The hospital houses medical, surgery, emergency, pediatric, and neonatal specialty ICUs.

Bacteria isolates and susceptibility

All the culture isolates were analyzed according to the hospital's standard protocol. Susceptibility data were obtained from the Department of Medical Laboratory Science's microbiology team. A broth microdilution method (BD Phoenix; Becton Dickinson; Sparks, MD, USA) was used for susceptibility testing from July 1st, 2018 to June 30th, 2019, and the isolates were classified as susceptible, resistant, or intermediate according to minimum inhibitory concentration (MIC) value and breakpoint from the 2017 Clinical and Laboratory Standards Institute (CLSI) guidelines for PA.[15] PTZ (Susceptible: MIC $\leq$16/4, intermediate: MIC = 32/4—64/4, resistant: MIC $\geq$128/4), ceftazidime (Susceptible: MIC $\leq$8, intermediate: MIC = 16, resistant:

MIC $\geq$32), cefepieme (Susceptible: MIC $\leq$8, intermediate: MIC = 16, resistant: MIC $\geq$32), meropenem (Susceptible: MIC $\leq$2, intermediate: MIC = 4, resistant: MIC $\geq$8), imipenem (Susceptible: MIC $\leq$2, intermediate: MIC = 4, resistant: MIC $\geq$8), gentamicin (Susceptible: MIC $\leq$4, intermediate: MIC = 8, resistant: MIC $\geq$16), amikacin (Susceptible: MIC $\leq$16, intermediate: MIC = 32, resistant: MIC $\geq$64), ciprofloxacin (Susceptible: MIC $\leq$1, intermediate: MIC = 2, resistant: MIC $\geq$4), levofloxacin (Susceptible: MIC $\leq$2, intermediate: MIC = 4, resistant: MIC $\geq$8). The intermediate isolates were considered resistant. The PA isolates isolated from ICU and from the general ward were categorized into the ICU and non-ICU groups, respectively.

Combination antibiogram susceptibility

The susceptibility to the antipseudomonal single antibiotics (PTZ, cefatazidime, cefepime, imipenem, or meropenem) or to those administered in combination with an aminoglycoside (gentamicin or amikacin) or fluoroquinolone (ciprofloxacin or levofloxacin) was calculated. An isolate's susceptibility to a combination regimen was defined as its susceptibility to at least one of the dual agents.

Statistics

The differences in susceptibility between the ICU and non-ICU isolates to single agents and to combination regimens were analyzed using the chi-square test. A p value of <0.05 was considered statistically significant. The Logistic Regression Analysis is indicated by p-values and odds ratios (ORs) with 95% confidence intervals (CIs). Statistical significant (two tail) is identified as p-value < 0.05. All analyses were conducted using IBM SPSS version 19.

Results

PA antimicrobial susceptibility to single antibiotics and combination regimens

A total of 880 PA isolates were included in our study from July 1st, 2018 to June 30th, 2019. The distribution of the 880 PA isolates was as following: 541 (61.5%) isolates collected from sputum, 191 (21.7%) from urine, 36 (4.09%) from wound, 32 (3.63%) from blood, 3 (0.34%) from bronchoalveolar lavage (BAL), 1 (0.11%) from cerebrospinal fluid (CSF) and 76 (8.63%) from other sample which included ascites, bile, pleural fluid, biopsy tissue or vagina. The susceptibility of all the isolates to the single β-lactam antibiotics ranged from 83.1% to 89.7% (Table 1). To expand the empirical coverage rate, we combined the β-lactam agents with aminoglycosides and fluoroquinolones (Table 1). The combination regimens containing amikacin provided the highest coverage rates ($\geq$98.9%) and those containing levofloxacin provided the lowest coverage rates (92.3%–94.1%) among all the combination regimens. The combination regimens containing ciprofloxacin provided slightly greater coverage than those containing levofloxacin but less coverage than those containing amikacin. All the combined regimens exhibited a greater coverage rate than did any of the single β-lactam antibiotics.

Risk factors for β-lactam agents resistant to PA isolates

To understand the risk factors to cause five β-lactam agents (ceftazidime, imipenem, cefepime, meropenem and PTZ) resistance, we use logistic regression analysis to test some risk factors included patient sex (male or female), species type (blood or other culture), isolated origin (ICU or Non-ICU). The results showed that ICU or Non-ICU origin isolates (ceftazidime: OR: 3.074, CI: 5.023–1.881, $p < 0.001$, imipenem: OR: 2.483, CI: 4.431–1.391, $p = 0.002$, cefepime: OR: 2.359, CI: 3.933–1.414, $p = 0.001$, meropenem: OR: 2.009, CI: 3.644–1.108, $p = 0.022$, PTZ: OR: 3.796, CI: 6.104–2.361, $p < 0.001$) was the independent risk factor for all five β-lactam agents resistant to PA isolates (Table 2).

Effect of ICU origin on susceptibility

We further investigated the differences in susceptibility to the single antibiotics and combination regimens between the ICU and non-ICU isolates. The coverage rates of the five β-lactam agents ranged from 85.6% to 90.7% and 61.5% to 82.4% in the non-ICU and ICU isolates, respectively (Table 3), indicating that the non-ICU isolates generally exhibited higher susceptibility to single antibiotics. We also conducted chi-square test to evaluate the differences in antibiotic susceptibility between the non-ICU and ICU isolates. The ICU isolates exhibited significantly lower

Table 1 Coverage rates of single antibiotics and combined regimens from July 1st 2018 to June 30th 2019 at Taipei Medical University Hospital.

	Monotherapy	With Gentamicin (85.5%)	With Amikacin (97.7%)	With Ciprofloxacin (83.8%)	With Levofloxacin (80.3%)
N = 880	(%)	(%)	(%)	(%)	(%)
Ceftazidime	83.8	94.2	99.0	94.7	93.8
Imipenem	84.4	93.6	99.3	93.5	92.3
Cefepime	89.7	96.3	99.1	94.7	94.1
Meropenem	89.3	96.4	99.1	94.9	93.9
Piperacillin/tazobactam	83.1	94.8	98.9	94.7	92.6

Table 2 Risk factors for β-lactam agents (ceftazidime, imipenem, cefepime, meropenem and PTZ) resistant to PA isolates with logistic regression analysis.

Covariate	Ceftazidime OR (95% CI)	*p*-value	Imipenem OR (95% CI)	*p*-value	Cefepime OR (95% CI)	*p*-value	Meropenem OR (95% CI)	*p*-value	PTZ OR (95% CI)	*p*-value
Patient sex (male or female)	1.067 (1.561, 0.729)	0.738	1.118 (1.769, 0.706)	0.634	1.117 (1.643, 0.760)	0.573	0.969 (1.510, 0.621)	0.889	1.110 (1.621, 0.761)	0.588
Species type (blood culture or other culture)	2.420 (2.420, 0.278)	0.721	0.306 (2.287, 0.041)	0.249	0.619 (2.085, 0.184)	0.438	0.965 (3.266, 0.285)	0.954	0.327 (1.405, 0.076)	0.133
ICU or Non-ICU origin	3.074 (5.023, 1.881)	<**0.001***	2.483 (4.431, 1.391)	**0.002***	2.359 (3.933, 1.414)	**0.001***	2.009 (3.644, 1.108)	**0.022***	3.796 (6.104, 2.361)	<**0.001***

PTZ: Piperacillin/tazobactam, ICU: Intensive care unit, OR: Odds ratio, CI: Confidence interval, *Means $p < 0.05$.

Table 3 Combination coverage rates for ICU and non-ICU isolates from July 1st 2018 to June 30th 2019 at Taipei Medical University Hospital.

Group	Non-ICU isolates ($N = 789$)					ICU isolates ($N = 91$)									
	Monotherapy (%)	With GEN (86.1%)	With AMI (97.6%)	With CIP (83.1%)	With LEV (80.0%)	Monotherapy (%)	*p*-value	With GEN (83.5%)	*p*-value	With AMI (98.9%)	*p*-value	With CIP (89.0%)	*p*-value	With LEV (83.5%)	*p*-value
Ceftazidime	85.7	94.8	98.9	94.7	93.8	67.0	<**0.001***	89.0	**0.025***	100	0.306	94.5	0.945	93.4	0.886
Imipenem	85.8	94.2	99.4	93.4	92.1	72.5	**0.002***	89.0	0.056	98.9	0.610	94.5	0.687	93.4	0.669
Cefepime	90.7	96.6	98.9	94.7	94.2	80.2	**0.001***	93.4	0.132	100	0.335	94.5	0.945	93.4	0.770
Meropenem	90.1	96.6	90.0	94.9	93.9	82.4	**0.024***	94.5	0.317	100	0.335	94.5	0.862	93.4	0.848
PTZ	85.6	95.6	98.9	94.8	93.0	61.5	<**0.001***	87.9	**0.002***	98.9	0.972	93.4	0.575	89.0	0.165

GEN: gentamicin, AMI: amikacin, CIP: ciprofloxacin, LEV: levofloxacin, PTZ: Piperacillin/tazobactam, ICU: Intensive care unit, *Means $p < 0.05$.

susceptibility to the ceftazidime—gentamicin ($p = 0.025$) and PTZ—gentamicin ($p = 0.002$) regimens.

Discussion

PA is the most common pathogen that causes nosocomial infections, particularly in the critically ill or immunocompromised patients.[1,16] In our study, ICU-origin isolated is the independent risk factor for five β-lactam agents' resistance. The ICU isolates were more resistant to the single β-lactam antibiotics than were the non-ICU isolates, and the single antibiotics' coverage rates of the ICU isolates ranged from 61.5% to 82.4%. This range was insufficient according to the most recent guideline, which mandate the hospitals should assure that 95% of patients with ventilator-associated pneumonia receive active empirical therapy in units where <10% of Gram-negative isolates are resistant to an agent being considered for monotherapy.[17] To this day, there is still no definite recommendation on optimal empirical coverage rate for all PA infection. For instance, in another study with oncology population at a single institution, effective combination coverage rate was defined as empirical coverage against ≥85% isolates.[18] In the absence of new β-lactam antibiotics, empirical combination regimens may be used to expand the coverage rate in the treatment of patients with critical bacterial infections.

The continuous use of combination therapy after isolation of a bacterial strain and collection of susceptibility data remains controversial because it may induce multidrug resistance in bacteria and because additive antibiotics may cause unnecessary side effects.[6,19,20] However, the available evidence indicates that the greatest benefit of combination regimen is its ability to increase the likelihood of selecting an effective antimicrobial agent for empirical therapy because MDR-GNB are associated with increased 30-days risk of mortality.[21] Empirical combination regimens have been used to treat some subgroups of patients, including those with invasive infections, neutropenia, or infections caused by PA.[22] Studies have suggested that adequate combination therapy reduces the mortality rate in patients with PA pneumonia.[3] A single-center study also revealed that inappropriate empirical therapy increased mortality among patients with PA blood stream infections (BSIs).[23] In Micek et al. study, inappropriate initial empirical antimicrobial treatment was associated with higher mortality among patients with PA BSIs and was more common in patient in who only received empirical monotherapy.[24]

In this study, we focused on the importance of determining appropriate empirical combination regimens to treat PA infections. Although local antibiograms were available to help us in selecting a proper empirical single β-lactam antibiotic in the hospital, the hospital did not yet have access to an antibiogram capable of assessing the appropriateness of local combination therapies. Combination therapies are developed with consideration given to the susceptibility of bacteria to the single antimicrobial agents used in the therapies, and antibiograms are useful clinical tools for evaluating the antimicrobial coverage of multiple antibiotics to inform physicians' decisions regarding optimal empirical combination therapies.[25] The combination antibiogram could provide more detail and accurate susceptibility information for clinician when they are trying to use empirical combination regiment to treat severe PA infection patients. For example, in our study, PTZ susceptibility to PA was 83.1% while expanded to 98.9% when amikacin was combined. Providing this information to clinicians could help them to determine optimal therapy regimen. Studies have reported that single antimicrobial agents exhibit low coverage of PA isolates; using such medications in combination with aminoglycosides (amikacin, gentamicin, or tobramycin) or fluoroquinolones (ciprofloxacin or levofloxacin) could significantly expand the coverage rates, and aminoglycosides can expand the coverage to a greater extent than can fluoroquinolones.[26,27] These results are consistent with those of our study; none of the single β-lactam antibiotics provided coverage of >85% of the ICU isolates. However, the addition of supplementary medications, especially amikacin, expanded the coverage rate of the treatments.

A previous study by Song et al. reported that tobramycin exhibited a significantly higher coverage rate of PA isolates when combined with ceftazidime, cefepime, aztreonam, piperacillin/tazobactam, meropenem, or imipenem than when combined with fluoroquinolones (ciprofloxacin and levofloxacin).[27] Smith et al. reported that an empirical combination of β-lactam antibiotics with aminoglycosides may be higher coverage to PA bacteremia in patients with cancer.[18] The adequate empirical combination therapy could reduce all-cause 28-day mortality in patients with bacteremic PA pneumonia.[28] Compared with adding fluoroquinolones, adding aminoglycodises to the β-lactam agents provides broadest coverage rate. In addition, previous studies highlighted the importance of adequate empirical therapy selection, as it was associated with lower mortality in patients with PA infections. Our results were similar to those of previously studies that reported that aminoglycosides, especially amikacin, could obtain the greatest coverage rate among the additive medications tested. The reason why constitutive overexpression of the active efflux system MexXY/OprM is a major cause of PA antibiotic resistance, including cefepime and fluoroquinolone coresistance,[29] might also explain why β-lactam antibiotics combined with aminoglycosides are more effective in treating PA infections than are fluoroquinolones alone. Furthermore, in our previous study, the use of fluoroquinolones, levofloxacin but not ciprofloxacin, appeared to be positive correlation with PA increased resistance.[30] To control the emergence of PA antibiotic resistance, the use of fluoroquinolones especially levofloxacin as the additive agents to β-lactam antibiotics must be avoided.

Studies have indicated that ICU stay is a risk factor of infection with resistant pathogenic strains.[31] To investigate differences in susceptibility to single agents and combination regimens between the ICU and non-ICU isolates, we conducted chi-square test. The non-ICU isolates exhibited significantly higher susceptibility to the β-lactam single antibiotics; however, although the β-lactam antibiotics were less effective in treating the ICU isolates, these isolates exhibited surprisingly high susceptibility to amikacin. All the amikacin combination regimens attained empirical coverage rates of ≥95%, which fulfills the stipulations detailed in the recent guideline.[17] Although all the combination regimens attained coverage rates of >85%; the ICU

isolates were significantly less susceptible to the PTZ—gentamicin regimen than to the other combination regimens. These findings indicate that amikacin empirical combination therapies might be more effective than the combination regimes containing gentamicin, ciprofloxacin, or levofloxacin.

Physicians should always consider the risk of nephrotoxicity and closely monitor patients' serum concentrations when prescribing aminoglycosides. Furthermore, intravenous administration of aminoglycosides results in poorer penetration of the lung than does the intravenous administration of fluoroquinolones, which makes using aminoglycosides to treat PA pneumonia difficult.[32,33] For these reasons, physicians may prefer to prescribe fluoroquinolones rather than aminoglycosides for the treatment of PA pneumonia.

Our study has several limitations. First, this study only evaluated the *in vitro* susceptibility of PA isolates; differences in clinical outcomes and mortality rates associated with empirical monotherapies and combination therapies were not evaluated in our study. Second, consensus on the definition of optimal empirical coverage has yet to be reached. Smith et al. argued that empirical coverage should be defined as effective bactericidal activity against $\geq$85% of isolates.[18] In our study, only the amikacin-containing combination regimens covered $\geq$95% of all the ICU and non-ICU isolates. Third, the ICU isolates might have mixed with ICU-admission or ICU-acquired isolates, which might exhibit different susceptibility to the single β-lactam antibiotics and combination regimens. Lastly, our study lacked experiment to investigate the mechanism of the difference combination regiments. Understanding the mechanism between different combinations can also help us to select optimal combination therapy.

Empirical combination therapy remains controversial today. However, some studies have reported that it might benefit some subgroups of patients, including those with critical MDR-PA infections.

Conclusion

To conclude our present study, local combination antibiogram should be developed to facilitate clinicians in selecting optimal combination regimen for critically infected patients caused by PA. Choosing aminoglycosides, especially amikacin, as the additive agents can maximize the empirical coverage rate.

Acknowledgements

This manuscript was edited by Wallace Academic Editing and the authors would like to acknowledge the Laboratory Animal Center at TMU for technical support in our experiment.

References

1. Moradali MF, Ghods S, Rehm BH. *Pseudomonas aeruginosa* lifestyle: a paradigm for adaptation, survival, and persistence. *Front Cell Infect Microbiol* 2017;**7**:39.
2. Zhang Y, Li Y, Zeng J, Chang YS, Han SH, Zhao J, et al. Risk factors for mortality of inpatients with Pseudomonas aeruginosa bacteremia in China: impact of resistance Profile in the mortality. *Infect Drug Resist* 2020;**13**:4115—23.
3. Paul M, Shani V, Muchtar E, Kariv G, Robenshtok E, Leibovici L. Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis. *Antimicrob Agents Chemother* 2010;**54**:4851—63.
4. Worthington RJ, Melander C. Overcoming resistance to β-Lactam antibiotics. *J Org Chem* 2013;**78**:4207—13.
5. Karaiskos I, Lagou S, Pontikis K, Rapti V, Poulakou G. The "Old" and the "New" antibiotics for MDR gram-negative pathogens: for whom, when, and how. *Front Public Health* 2019;**7**:151.
6. Hirsch EB, Tam VH. Impact of multidrug-resistant *Pseudomonas aeruginosa* infection on patient outcomes. *Expert Rev Pharmacoecon Outcomes Res* 2010;**10**:441—51.
7. Schmid A, Wolfensberger A, Nemeth J, Schreiber PW, Sax H, Kuster SP. Monotherapy versus combination therapy for multidrug-resistant gram-negative infections: systematic review and meta-analysis. *Sci Rep* 2019;**9**:15290.
8. Tsai WC, Syue LS, Ko WC, Lo CL, Lee NY. Antimicrobial treatment of monomicrobial phenotypic carbapenem-resistant Klebsiella pneumoniae bacteremia: two are better than one. *J Microbiol Immunol Infect* 2021. https://doi.org/10.1016/j.jmii.2021.09.002.
9. Leekha S, Terrell CL, Edson RS. General principles of antimicrobial therapy. *Mayo Clin Proc* 2011;**86**:156—67.
10. Gudiol C, Tubau F, Calatayud L, Garcia-Vidal C, Cisnal M, Sánchez-Ortega I, et al. Bacteraemia due to multidrug-resistant Gram-negative bacilli in cancer patients: risk factors, antibiotic therapy and outcomes. *J Antimicrob Chemother* 2011;**66**:657—63.
11. Micek ST, Lloyd AE, Ritchie DJ, Reichley RM, Fraser VJ, Kollef MH. Pseudomonas aeruginosa bloodstream infection: importance of appropriate initial antimicrobial treatment. *Antimicrob Agents Chemother* 2005;**49**:1306—663.
12. Kang CI, Kim SH, Kim HB, Park SW, Choe YJ, Oh MD, et al. Pseudomonas aeruginosa bacteremia: risk factors for mortality and influence of delayed receipt of effective antimicrobial therapy on clinical outcome. *Clin Infect Dis* 2003;**37**:745—51.
13. Bhat S, Fujitani S, Potoski BA, Capitano B, Linden PK, Shutt K, et al. Pseudomonas aeruginosa infections in the Intensive Care Unit: can the adequacy of empirical beta-lactam antibiotic therapy be improved? *Int J Antimicrob Agents* 2007;**30**:458—62.
14. Thompson RZ, Sargel CL, Moore-Clingenpeel M, Karsies TJ. Creation of a combination antibiogram for Pseudomonas aeruginosa in a pediatric intensive care unit. *J Pediatr Pharmacol Therapeut* 2021;**26**:828—33.
15. CLSI. Performance standards for antimicrobial susceptibility testing. In: *CLSI supplement M100*. 27th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
16. Lin KY, Lauderdale TL, Wang JT, Chang SC. Carbapenem-resistant *Pseudomonas aeruginosa* in Taiwan: prevalence, risk factors, and impact on outcome of infections. *J Microbiol Immunol Infect* 2016;**49**:52—9.
17. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LBM, et al. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the infectious diseases society of America an3d the American Thoracic Society. *Clin Infect Dis* 2016;**63**:e61—111.
18. Smith ZR, Tajchman SK, Dee BM, Bruno JJ, Qiao W, Tverdek FP. Development of a combination antibiogram for *Pseudomonas aeruginosa* bacteremia in an oncology population. *J Oncol Pharm Pract* 2016;**22**:409—15.

19. Worthington RJ, Melander C. Combination Approaches to combat multi-drug resistant bacteria. *Trends Biotechnol* 2013; **31**:177—84.
20. Saputra EC, Huang L, Chen Y, Tucker-Kellogg L. Combination therapy and the evolution of resistance: the theoretical merits of synergism and antagonism in cancer. *Cancer Res* 2018;**78**: 2419—31.
21. Patolia S, Abate G, Patel N, Patolia S, Frey S. Risk factors and outcomes for multidrug-resistant Gram-negative bacilli bacteremia. *Ther Adv Infect Dis* 2018;**5**:11—8.
22. Tamma PD, Cosgrove SE, Maragakis LL. Combination therapy for treatment of infections with gram-negative bacteria. *Clin Microbiol Rev* 2012;**25**:450—70.
23. Retamar P, Portillo MM, López Prieto MD, Rodríguez López F, de Cueto M, Garcia MVR, et al. Impact of inadequate empirical therapy on the mortality of patients with bloodstream infections: a propensity score-based analysis. *Antimicrob Agents Chemother* 2012;**56**:472—8.
24. Micek ST, Lloyd AE, Ritchie DJ, Reichley RM, Fraser VJ, Kollef MH. Pseudomonas aeruginosa bloodstream infection: importance of appropriate initial antimicrobial treatment. *Antimicrob Agents Chemother* 2005;**49**:1306—11.
25. Tamma PD, Cosgrove SE. Antimicrobial stewardship. *Infect Dis Clin* 2011;**25**:245—60.
26. Mizuta M, Linkin DR, Nachamkin I, Fishman NO, Weiner MG, Sheridan A, et al. Identification of optimal combinations for empirical dual antimicrobial therapy of *Pseudomonas aeruginosa* infection: potential role of a Combination Antibiogram. *Infect Control Hosp Epidemiol* 2006;**27**:413—5.
27. Song M, Dilworth TJ, Munson E, Davis JJ, Elshaboury RH. Results of a local combination therapy antibiogram for *Pseudomonas aeruginosa* isolates: is double worth the trouble? *Ther Adv Infect Dis* 2017;**4**:165—70.
28. Park SY, Park HJ, Moon SM, et al. Impact of adequate empirical combination therapy on mortality from bacteremic Pseudomonas aeruginosapneumonia. *BMC Infect Dis* 2012;**12**: 308.
29. Dupont P, Hocquet D, Jeannot K, Chavanet P, Plésiat P. Bacteriostatic and bactericidal activities of eight fluoroquinolones against MexAB-OprM-overproducing clinical strains of *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 2005;**55**:518—22.
30. Lee YJ, Liu HY, Lin YC, Sun KL, Chun CL, Hsueh PR. Fluoroquinolone resistance of *Pseudomonas aeruginosa* isolates causing nosocomial infection is correlated with levofloxacin but not ciprofloxacin use. *Int J Antimicrob Agents* 2010;**35**: 261—4.
31. Tan R, Liu J, Li M, Huang J, Sun J, Qu H. Epidemiology and antimicrobial resistance among commonly encountered bacteria associated with infections and colonization in intensive care units in a university-affiliated hospital in Shanghai. *J Microbiol Immunol Infect* 2014;**47**:87—94.
32. Pennington JE, Reynolds HY. Concentrations of gentamicin and carbenicillin in bronchial secretions. *J Infect Dis* 1973;**128**: 63—8.
33. Ratjen F, Brockhaus F, Angyalosi G. Aminoglycoside therapy against *Pseudomonas aeruginosa* in cystic fibrosis: a review. *J Cyst Fibros* 2009;**8**:361—9.