

Association Between Minimum Inhibitory Concentration, Beta-lactamase Genes and Mortality for Patients Treated With Piperacillin/Tazobactam or Meropenem From the MERINO Study

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Introduction. This study aims to assess the association of piperacillin/tazobactam and meropenem minimum inhibitory concentration (MIC) and beta-lactam resistance genes with mortality in the MERINO trial.

Methods. Blood culture isolates from enrolled patients were tested by broth microdilution and whole genome sequencing at a central laboratory. Multivariate logistic regression was performed to account for confounders. Absolute risk increase for 30-day mortality between treatment groups was calculated for the primary analysis (PA) and the microbiologic assessable (MA) populations.

Results. In total, 320 isolates from 379 enrolled patients were available with susceptibility to piperacillin/tazobactam 94% and meropenem 100%. The piperacillin/tazobactam nonsusceptible breakpoint (MIC >16 mg/L) best predicted 30-day mortality after accounting for confounders (odds ratio 14.9, 95% confidence interval [CI] 2.8–87.2). The absolute risk increase for 30-day mortality for patients treated with piperacillin/tazobactam compared with meropenem was 9% (95% CI 3%–15%) and 8% (95% CI 2%–15%) for the original PA population and the post hoc MA populations, which reduced to 5% (95% CI –1% to 10%) after excluding strains with piperacillin/tazobactam MIC values >16 mg/L. Isolates coharboring extended spectrum β -lactamase (ESBL) and OXA-1 genes were associated with elevated piperacillin/tazobactam MICs and the highest risk increase in 30-day mortality of 14% (95% CI 2%–28%).

Conclusions. After excluding nonsusceptible strains, the 30-day mortality difference from the MERINO trial was less pronounced for piperacillin/tazobactam. Poor reliability in susceptibility testing performance for piperacillin/tazobactam and the high

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prevalence of OXA coharboring ESBLs suggests that meropenem remains the preferred choice for definitive treatment of ceftriaxone nonsusceptible *Escherichia coli* and *Klebsiella*.

Keywords. piperacillin-tazobactam; meropenem; extended spectrum beta-lactamase; bloodstream infection.

Resistance to 3rd generation cephalosporins (3GC) due to extended spectrum β -lactamases (ESBL) in Enterobacterales among bloodstream infections (BSIs) has become increasingly commonplace [1]. Although carbapenems are considered the gold standard for treatment of ESBL-producing Enterobacterales (ESBL-PE), avoidance of carbapenems has been seen as a key tool in antimicrobial stewardship practice [2–4].

However, treatment of ESBL-PE BSI has significantly varied due to contradictory results among clinical studies [5–8]. The results of the MERINO trial (NCT02176122/ACTRN12613000532707) did not support the use of piperacillin-tazobactam (PTZ) for the treatment of BSI caused by ceftriaxone nonsusceptible *Escherichia coli* or *Klebsiella pneumoniae*, with higher 30-day mortality observed in patients randomized to PTZ compared to meropenem (MER) [9–10]. We therefore performed a post hoc analysis based upon minimum inhibitory concentration (MIC) testing and resistance genes detected by whole genome sequencing (WGS) comparative to the 30-day mortality of patients treated with PTZ and MER.

METHODS

Collection of Isolates

The first positive blood culture isolate was collected from enrolled patients at each locally participating study site before being transferred to and stored centrally at the University of Queensland, Centre for Clinical Research (UQCCR) for antimicrobial susceptibility testing and whole genome sequencing. For the purposes of this analysis, we included patients from the MERINO trial from whom the first positive blood culture isolate was received, were randomized appropriately, received at least 1 dose of the study drug and were assessable for 30-day mortality. This group is referred to as the “microbiologic assessable population” in this study.

Antimicrobial Susceptibility Testing

Custom made sensititer plates sourced from Thermo Fisher Scientific were used to perform broth microdilution (BMD) in batch testing according to manufacturer’s instructions with the final inoculum concentration at 5×10^5 . Tazobactam concentration was kept constant at 4 mg/L and piperacillin concentration ranged from 1 to 64 mg/L, whereas meropenem concentration ranged from 0.015 to 16 mg/L. Purity plates and colony count plates were prepared on 5% horse blood Columbia agar and incubated with the 96-well sensititer plates at 37°C in aerobic conditions for 18–24 hours. Plates were read using the sensititer manual viewer. The MIC was interpreted as the lowest

concentration of an antimicrobial that inhibits visual growth. Only plates with a positive growth control and that passed quality control for purity and colony counts were recorded. Quality control for the sensititer plates was performed routinely during the post-hoc study using *E. coli* ATCC25922 and *K. pneumoniae* ATCC700603 strains.

As a higher than expected rate of nonsusceptibility to PTZ was found when the isolates were tested post hoc by broth microdilution, further details on testing methods were sought for each enrolling site. Although laboratories were unable to retrospectively provide details on individual susceptibility testing results for each isolate, most laboratories reported using Vitek 2 or disk diffusion for susceptibility testing in the trial. As such, we then performed further post hoc PTZ susceptibility testing using the 2 predominant methods reportedly used: Vitek 2 AST-N247 (BioMérieux, France) and disk diffusion according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations (PTZ disk 30–6 μ g, Oxoid) at Pathology Queensland, Brisbane, Australia [11].

Isolates were assessed as susceptible based on EUCAST (v9.0) and Clinical and Laboratory Standards Institute (CLSI) (M100-ED29:2019) breakpoint tables [11, 12]. Isolates with PTZ MIC ≤ 16 mg/L were determined as susceptible by EUCAST breakpoints given the standard dose used in the MERINO trial was 4.5g Q6H (the recommended high dose for piperacillin/tazobactam when isolates test as susceptible, increased exposure). Clinical breakpoints for PTZ differ between EUCAST and CLSI predominantly for the resistant breakpoint (EUCAST S ≤ 8 mg/L and susceptible, increased exposure ≤ 16 mg/L, R > 16 mg/L; CLSI S ≤ 16 mg/L, R > 64 mg/L) with differences between the susceptible breakpoint related to the higher doses recommended for patients due to infections in Enterobacterales with PTZ MICs of 16 mg/L by EUCAST [12, 13]. Rates of very major error (susceptible by Vitek 2 or disk diffusion but resistant by broth microdilution), major error (resistant by Vitek 2 or disk diffusion but susceptible by broth microdilution) and minor error (isolates within the area of technical uncertainty or intermediate category but susceptible or resistant by broth microdilution) were calculated.

Cohen kappa statistic was calculated to measure agreement between categories based on BMD testing compared with Vitek 2 AST and disk diffusion. Calculated kappa values of ≤ 0.40 are considered to reflect poor categorical agreement, with those between > 0.40 and ≤ 0.80 demonstrating moderate to substantial agreement and those > 0.80 reflecting near perfect categorical agreement.

Whole Genome Sequencing

DNA extraction and whole genome sequencing were performed as previously described and can be found in further detail in the [Supplementary section](#) [10]. Individual genes were reviewed and characterized by their corresponding beta-lactamase enzymes and distinctive substrates using the Bush-Jacoby-Medeiros classification scheme [14]. Beta-lactamase genes were grouped as ESBL, plasmid-mediated *ampC*, and narrow-spectrum OXA. Group 2b enzymes such as TEM-1 and SHV-1, those that hydrolyse penicillins and early cephalosporins, were not included as they do not have any effect on PTZ activity unless hyperproduced in the setting of upstream promoters [14, 15]. No carbapenemase genes or group 2br or 2ber enzymes were identified—the latter confer resistance to beta-lactamase inhibitors.

Statistics

To determine susceptibility breakpoints, binary recursive partitioning methodology, classification and regression tree (CART) modeling was performed. In particular, CART was used to determine an appropriate breakpoint in PTZ MIC that maximized the difference in 30-day mortality between low and high PTZ MICs. To determine the smallest tree with the lowest misclassification loss, pruning and a 10-fold cross-validation were used in the CART analysis, performed using *R*: a language and environment for statistical computing.

Bivariate logistic regression of \log_2 transformed MIC and MIC >16 $\mu\text{g/mL}$ (dichotomous variable) was performed to look for association with 30-day mortality in patients treated with PTZ. As the \log_2 transformed PTZ MIC was not statistically associated with 30-day mortality, PTZ MIC >16 $\mu\text{g/mL}$ was used in a backward stepwise multivariable logistic regression model with other potential predictors (identified in [Table 1](#)) of 30-day mortality in the PTZ arm. A second CART analysis of PTZ MIC with 30-day mortality was then performed using the additional variables identified in the multivariate model as statistically significant predictor variables ($P < .2$) of 30-day mortality using the same method as described above ([Supplementary Figure 3](#)).

A comparison of 30-day mortality by treatment group was performed in order to calculate the absolute risk increase for

treatment with PTZ compared with MER [16, 17]. We included 2 subpopulations based on broth microdilution MICs; the first sub-population excludes any patient with nonsusceptible strains (PTZ MIC >16 mg/L; MER MIC >1 mg/L CLSI, or MIC >2 mg/L EUCAST) in either intervention arm—this reflects the intended inclusion/exclusion criteria of the MERINO study. The second subpopulation excludes those that had isolates that were resistant to either MER (MIC >2 mg/L CLSI; MIC >8 mg/L EUCAST) or PTZ (MIC >16 mg/L EUCAST; MIC >64 CLSI)—thereby reflecting isolates with susceptible and intermediate categories. Absolute risk increase was also compared for PTZ and MER stratified by beta-lactam resistome as characterized by WGS.

RESULTS

A total of 320 primary blood culture isolates (278 *E. coli* and 42 *K. pneumoniae*) from 379 enrolled patients (84%) were included in the post hoc microbiologic assessable population, with 163/191 (85%) from the MER arm and 157/187 (84%) from the PTZ arm available for this analysis. The isolates available from all enrolled patients grouped by country were: Singapore 139/155, Australia 76/85, New Zealand 19/19, Canada 2/2, South Africa 8/11, Italy 6/25, Turkey 39/46, Lebanon 12/15, and Saudi Arabia 19/22. Of the patients included in the microbiologic assessable population, 18 (18/157, 11%) deaths occurred in the PTZ arm compared to 6 (6/163, 4%) deaths in the MER arm. Of the isolates not recovered for this post hoc analysis from enrolled patients, there were 5 deaths in 30 patients in the PTZ arm and 1 death in 28 patients in the MER arm. MIC distributions for PTZ and MER from this population are shown in [Figure 1](#) (stratified by intervention arm).

Beta-lactamase Genes

The predominant beta-lactamase genes with activity against 3GCs identified by WGS belonged to the CTX-M group (177 *bla*_{CTX-M-15}, 42 *bla*_{CTX-M-27}, 36 *bla*_{CTX-M-14}, 12 *bla*_{CTX-M-55}, 3 *bla*_{CTX-M-3}, 1 *bla*_{CTX-M-24}, 1 *bla*_{CTX-M-134}, 1 *bla*_{CTX-M-174}), whereas a further 12 isolates were identified as harboring SHV ESBL genes (8 *bla*_{SHV-106}, 2 *bla*_{SHV-12}, 2 *bla*_{SHV-27}). In addition, 36 isolates harbored plasmid-encoded *ampC* genes (25 *bla*_{CMY-2}, 2 *bla*_{CMY-42}, 2 *bla*_{CMY-138}, 1 *bla*_{CMY-146}, 6 *bla*_{DHA-1}) and 3 *E. coli* isolates were identified with chromosomal mutations in *ampC* promoter/attenuator genes resulting in predicted overexpression and consistent with *ampC* upregulation ([Supplementary Figure 1](#)) [18].

Narrow spectrum oxacillinase (OXA) genes were found in 102 isolates and included 99 OXA-1, 1 OXA-9, and 2 OXA-10. Almost all OXA genes (OXA-1, OXA-9, and OXA-10) were found in isolates coharboring the CTX-M-15 enzyme (96%). The OXA/CTX-M-15 combination was geographically distributed among all regions: Middle East (45%); Turkey/Mediterranean Europe region (43%); South Africa (38%) and Singapore (33%); Australia, New Zealand, and Canada combined (21%). No OXA

Table 1. Logistic Regression Model for Assessment of 30-day Mortality for Patients Treated With Piperacillin/Tazobactam

Variable	Bivariate Analysis		Multivariate Analysis	
	OR	P	aOR	P
Log ₂ (MIC)	1.2 (0.9–1.6)	.20	...	
MIC > 16 mg/L	10.3 (2.6–41.9)	<.001	14.9 (2.8–87.2)	.002
UTI source	0.4 (0.2–1.1)	.09	0.6 (0.2–1.8)	.3
Charlson comorbidity score	1.6 (1.3–2.0) ^a	<.001	1.7 (1.3–2.2) ^a	<.001

Abbreviations: aOR, adjusted odds ratio; MIC, minimum inhibitory concentration; UTI, urinary tract infection.

^aCalculated for each numerical increase in Charlson Comorbidity Score.

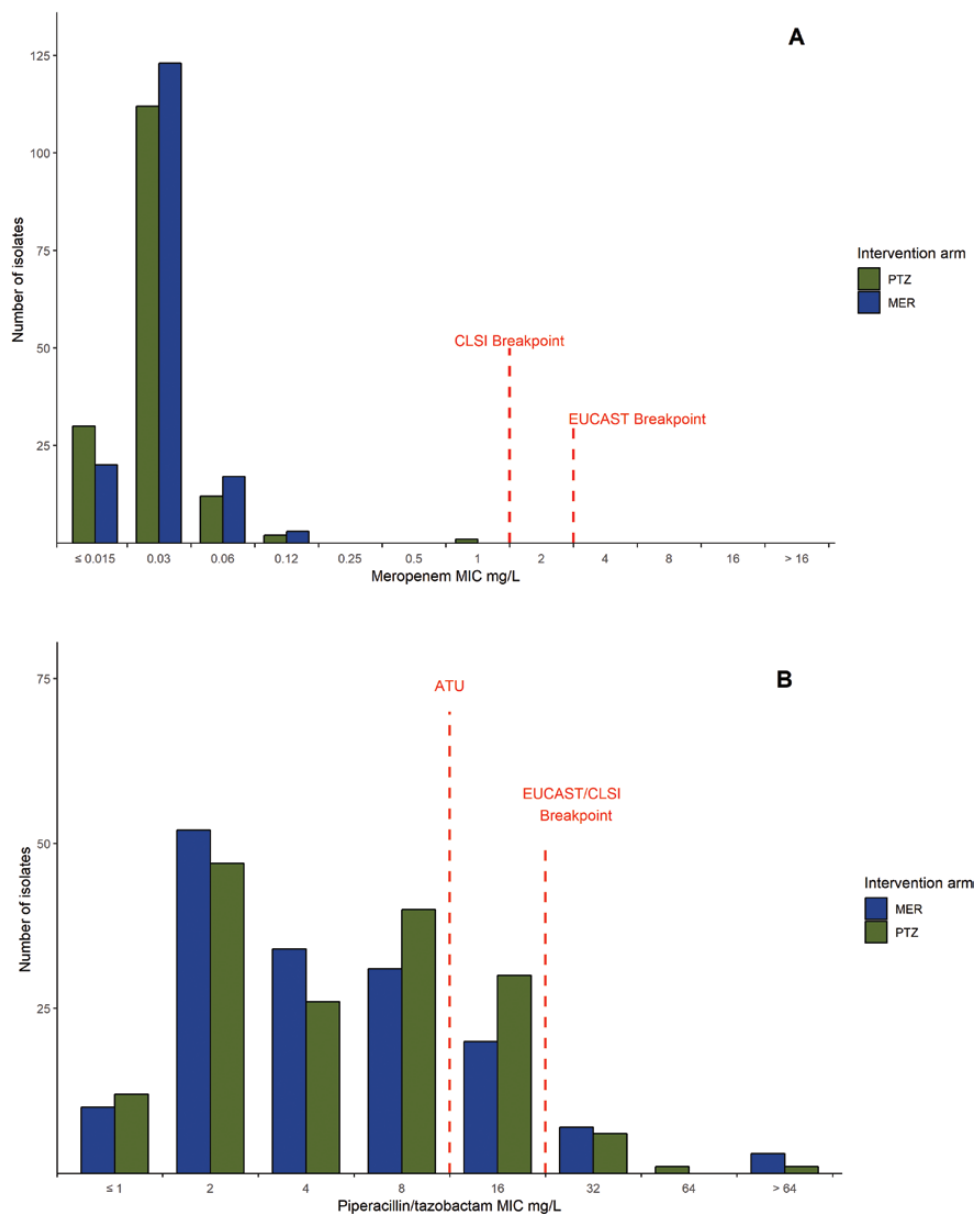


Figure 1. A, B: Distribution of MICs. Abbreviations: ATU, area of technical uncertainty; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MER, meropenem; MIC, minimum inhibitory concentration; PTZ, piperacillin/tazobactam.

genes encoding carbapenemases (eg. OXA-48 and related enzymes) were found.

In vitro Susceptibility

A strict inclusion criterion for enrolment in the MERINO trial was susceptibility of isolates to both PTZ and MER as performed at the recruiting sites clinical microbiology laboratory. However, in this post hoc microbiologic assessable population when analyzed using a reference broth microdilution method, overall susceptibility to PTZ and MER were 94% and 100% using EUCAST breakpoints (PTZ: susceptible ≤ 8 mg/L, susceptible increased exposure > 8 mg and ≤ 16 mg/L, area of technical uncertainty > 8 mg/L and ≤ 16 mg/L, and resistant > 16 mg/L; MER

susceptible ≤ 2 mg/L, susceptible increased exposure ≤ 8 mg/L, and resistant > 8 mg/L) For CLSI breakpoints, PTZ and MER susceptibility rates were 94% and 100% susceptible (PTZ: susceptible ≤ 16 mg/L, intermediate > 16 mg and ≤ 64 mg/L and resistant > 64 mg/L; MER: susceptible ≤ 1 mg/L, intermediate > 1 mg/L and ≤ 2 mg/L, and resistant > 2 mg/L). Of the isolates that tested susceptible to PTZ, 17% had MIC values within the area of technical uncertainty category. The very major error and major error rates for PTZ by Vitek 2 were higher for EUCAST than CLSI breakpoints (67% vs 0% and 5% vs 0%, respectively). Using the area of technical uncertainty and intermediate category, minor error rates were also higher for EUCAST than CLSI (17% vs 9%). The overall essential

agreement between Vitek 2 and broth microdilution was 87%. The very major error, major error and minor error rates for disk diffusion were 44%, 1%, and 19%, respectively (Figure 6).

Mean kappa values and 95% confidence intervals (CI) for PTZ AST by Vitek 2 compared with BMD were low for both CLSI breakpoints (0.16; 0.018 to 0.34) and EUCAST breakpoints (0.27; 0.16 to 0.38) as well as for disk diffusion compared with BMD using EUCAST breakpoints and disk concentration (0.34; 0.23 to 0.45).

Isolates harboring narrow spectrum oxacillinase (OXA) genes in addition to ESBL genes had significantly higher modal PTZ MICs than those with only ESBLs alone (8 mg/L vs 2 mg/L; Figure 2, $P < .001$).

Sequence Types

A diverse variety of sequence types (ST) were also identified from sequencing; however, the most common strain type was ST131 *E. coli* (143 isolates) and of this ST, 45% coharbored OXA genes, which was higher than the rate among the other combined STs (21%). ST131 *E. coli* coharboring $bla_{OXA-1/CTX-M-15}$ had an elevation in modal PTZ MICs to 8 mg/L (range 2 mg/L to 32 mg/L) compared to ST131 *E. coli* isolates only harboring $bla_{CTX-M-15}$, which had modal MICs of 2 mg/L (range 1 mg/L to 16 mg/L), reflecting an identical distribution for the bla_{OXA-1} presence or absence on PTZ MICs found for the other combined ST.

Relationship of Mortality by MIC

Figure 3A and B and Supplementary Tables 2 and 3 show the 30-day mortality rate by MIC for both respective intervention arms. Figure 4 demonstrates the absolute risk increase of

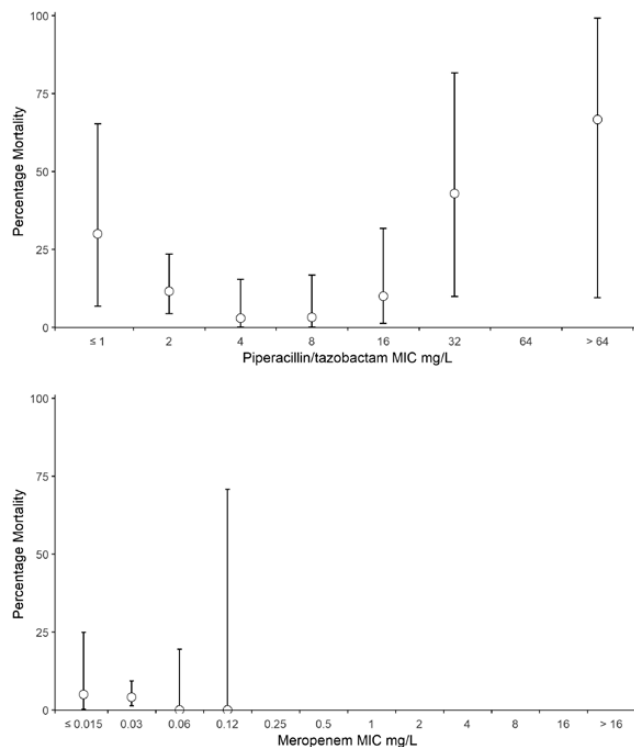


Figure 3. A, Percent mortality piperacillin/tazobactam. B, Percent mortality meropenem. Abbreviation: MIC, minimum inhibitory concentration.

30-day mortality for PTZ comparative to MER stratified by subpopulations. The between group mortality difference was less pronounced than for the original MERINO trial analysis, when PTZ nonsusceptible strains were excluded. This effect was noticed even further when isolates within the PTZ area of

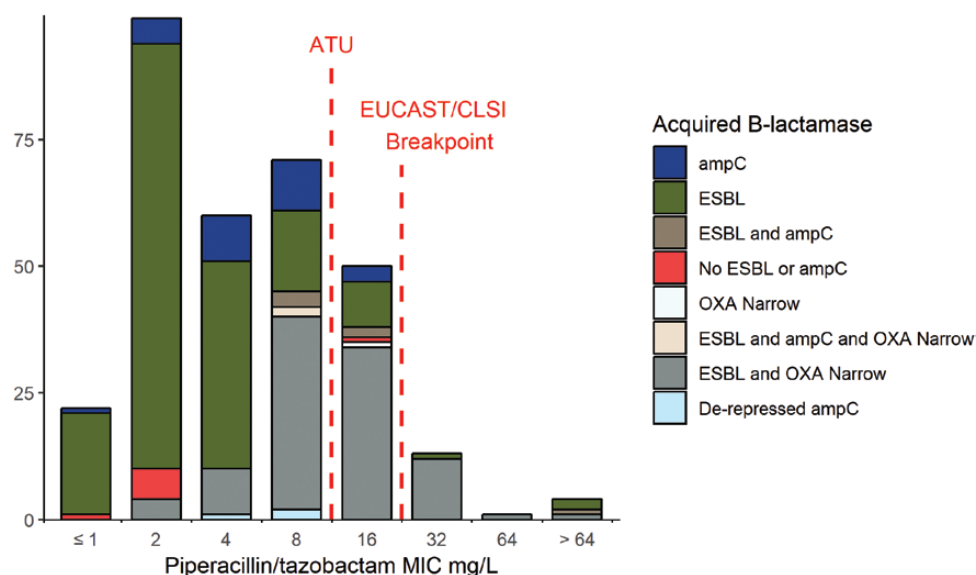


Figure 2. Piperacillin/Tazobactam resistome. Abbreviations: ATU, area of technical uncertainty; CLSI, Clinical and Laboratory Standards Institute; ESBL, extended spectrum β -lactamase; MIC, minimum inhibitory concentration.

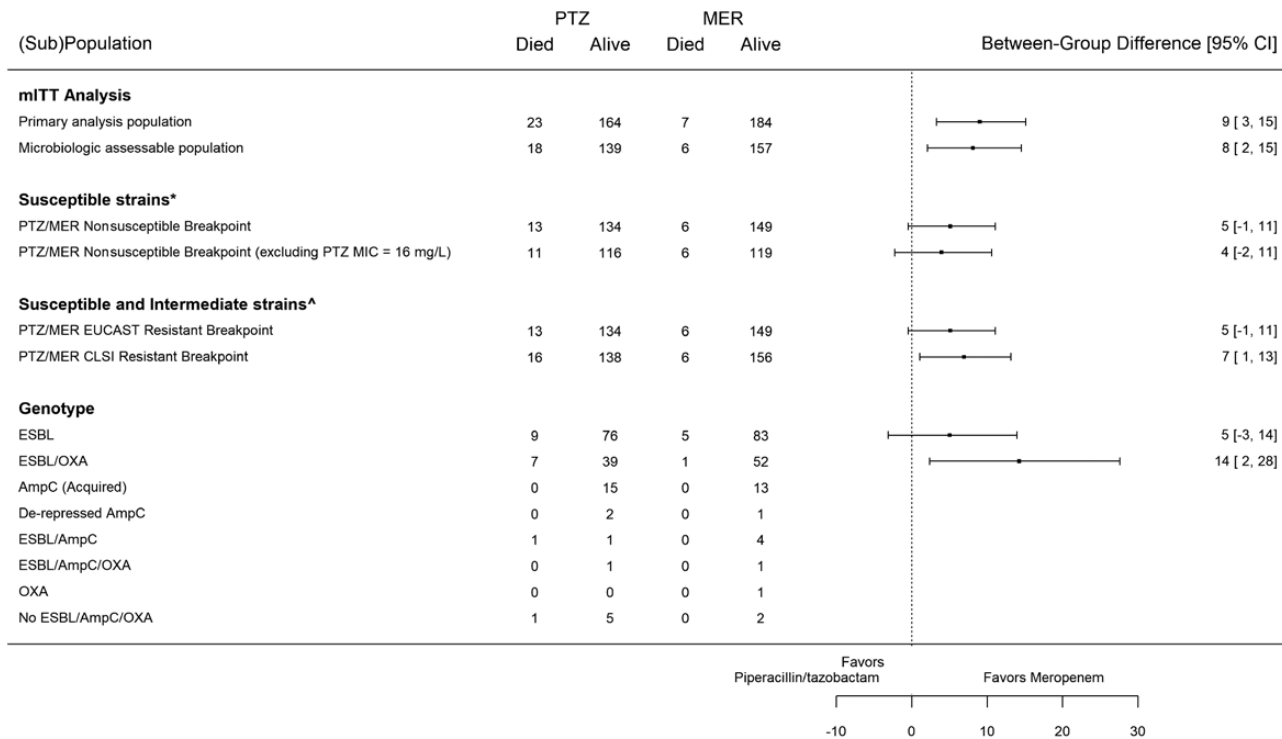


Figure 4. Meropenem Forrest plot. Abbreviations: CI, confidence interval; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MER, meropenem; MIC, minimum inhibitory concentration; PTZ, piperacillin/tazobactam.

technical uncertainty (MIC = 16 mg/L) were excluded, or where the analysis was performed for ESBL only strains. Furthermore, CART demonstrated an association between 30-day mortality and isolates with PTZ MICs >16 mg/L for patients treated with PTZ (Figure 5), whereas for MER, no statistically significant MIC cutoff was associated with 30-day mortality.

Supplementary Table 1 presents patient demographics and risk factors for MER and PTZ arms for the patients included in this post hoc analysis.

In Table 1, a significant association (odds ratio 10.3, 95% CI 2.6–41.9) was found for 30-day mortality with MIC values >16 mg/L and remained significant once adjusted for UTI

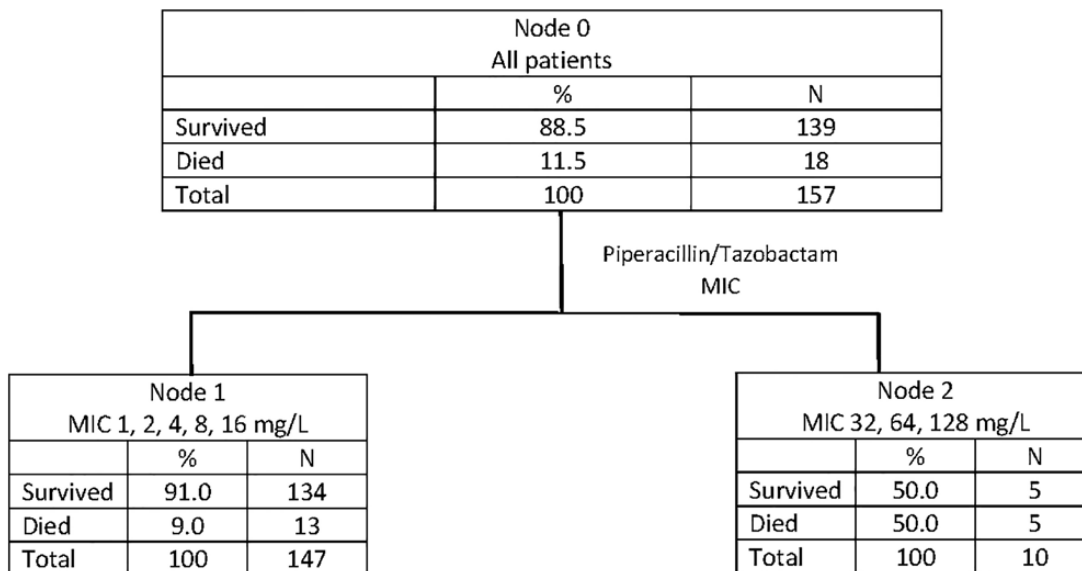


Figure 5. Classification and regression tree (CART). Abbreviation: MIC, minimum inhibitory concentration.

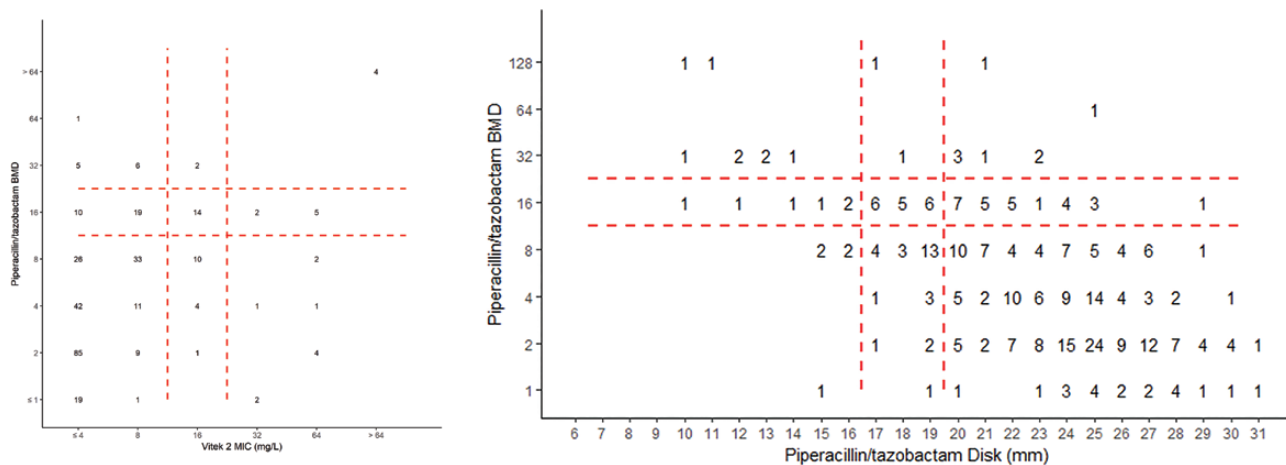


Figure 6. A, B: Piperacillin/tazobactam figures. Abbreviations: BMD, broth microdilution; MIC, minimum inhibitory concentration.

source and Charlson comorbidity (odds ratio 14.9, 95% CI 2.8–87.2).

DISCUSSION

The results of the MERINO trial did not support the use of PTZ for the treatment of bloodstream infections caused by ceftriaxone nonsusceptible Enterobacteriales [10]. Since publication, there has been significant discussion as to potential explanations for mortality differences between both arms [10, 19, 20]. In this study, we found an unexpectedly high rate of nonsusceptibility to PTZ using broth microdilution compared to Vitek or disk diffusion, as well as a significant 30-day mortality difference for patients treated with PTZ at a breakpoint of >16mg/L compared with ≤16 mg/L. The nonsusceptibility rate by broth microdilution differed substantially from the analysis in the MERINO trial publication where gradient MIC test strips (BioMerieux and Liofilchem) were used [10, 21]. As such, in the trial publication, there was no relationship initially reported between MIC values and 30-day mortality, which differs from the findings of this post hoc analysis [10].

An important difference between PTZ and MER MIC distributions was also noted in this study: almost all isolates had MER MIC values within the wild-type distribution and subsequently were at least 2–3 dilutions lower than the susceptible clinical breakpoint. However, the PTZ MIC distribution was up to and crossing the susceptible clinical breakpoint. As such, the performance of automated PTZ susceptibility testing using systems such as the Vitek 2 in the MERINO trial likely had a significant effect on 30-day mortality difference between MER and PTZ through the inclusion of patients with isolates that were nonsusceptible to PTZ.

After reanalysis of 30-day mortality for patients with PTZ susceptible isolates by EUCAST and CLSI breakpoints, the absolute risk increase for the PTZ group compared to MER was

attenuated. Using clinical breakpoints and examining only PTZ susceptible isolates, the between group difference in mortality was 5% (95% CI –1% to 10%). and excluding also those with MICs within the area of technical uncertainty, was 4% (95% CI –3% to 10%). Although these findings suggest PTZ may be a reliable option for PTZ susceptible strains, it should therefore be emphasized that at present, MER remains a safer choice for treatment of patients with bloodstream infections due to ceftriaxone nonsusceptible strains owing to performance issues with PTZ susceptibility testing.

A significant proportion of isolates collected for this analysis harbored OXA enzymes, with associated elevations in the modal MIC of PTZ compared to isolates with ESBLs alone from 2 mg/L to 8 mg/L, which was also recently described by Livermore et al [22]. The greatest absolute risk increase for 30-day mortality between PTZ and MER was seen in isolates coharboring ESBL and OXA genes. The detection of narrow spectrum OXA genes may therefore be of help in predicting possible PTZ nonsusceptibility and worse outcomes for PTZ treatment.

We found by CART analysis that a PTZ MIC of >16 mg/L or ≤16 mg/L was the most appropriate MIC breakpoint associated with 30-day mortality. Previous studies have looked at associations of clinical outcomes with PTZ MICs with contrasting results, likely related to heterogeneity amongst the source of infection [23–25]. This finding would suggest that the current susceptible breakpoint for CLSI and EUCAST of ≤16 mg/L is appropriate, but that the resistant breakpoint set by EUCAST (R >16 mg/L) is more appropriate than the CLSI breakpoint (R >64 mg/L).

The clinical implication of this post hoc study is concerning in light of the original MERINO trial given this current study demonstrates significant issues with performance of PTZ susceptibility testing in clinical microbiology laboratories and the high prevalence of OXA-harboring strains among ESBLs. It

remains to be determined whether these concerns can be ameliorated by more reliable susceptibility testing, possibly combined with rapid characterization of beta-lactamase gene content.

There are several limitations to our study. In particular, we were not able to collect all initial isolates from enrolled patients, although the number of isolates not available from each arm appeared to be equivalent. However, the mortality rate in the nonavailable isolates from the PTZ arm was higher than the rate among available isolates (16.7% vs 11.5%), and it is possible that this may have influenced the outcomes from this analysis. In addition, we were unable to perform a Pharmacokinetic/Pharmacodynamic (Pk/Pd) analysis to determine the relationship between PTZ exposure and 30-day mortality. However, as the trial stipulated a 30-minute infusion of PTZ every 6 hours (adjusted for renal function), we would suggest that dosing for PTZ was optimized according to current evidence and would be likely to have achieved appropriate piperacillin target attainment for MIC values up to 16 mg/L [26]. Recent publications, however, have suggested that tazobactam Pk-Pd target indexes may be suboptimal for current PTZ dosing strategies [27].

In conclusion, we identified a strong association between PTZ MIC and mortality in the MERINO trial. A higher than expected rate of nonsusceptibility to PTZ was identified by broth microdilution and MIC >16 mg/L was related to greater 30-day mortality. Significant error rates were identified in PTZ AST that account for some of the difference in mortality between MER and PTZ treated groups. These findings would suggest caution in using PTZ for bloodstream infections caused by ceftriaxone nonsusceptible *E. coli* and *Klebsiella* spp. and indicate that clinical microbiology laboratories may wish to perform further assessment of current PTZ susceptibility testing for these organisms.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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