Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2023 doi:10.20944/preprints202305.0858.v1

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Mortality due to Multi-drug resistant gram negative bacteremia in an endemic region: No better than toss a coin

Olga Tsachouridou[1*], Dimitrios Pilalas[1], Sideris Nanoudis[1], Athanasios Antoniou[1],Isidora Bakaimi[1], Theofilos Chrysanthidis[1], Konstantinos Markakis[1], Angeliki Kassomenaki[2], Paraskevi Mantzana[2], Efthymia Protonotariou[2], Lemonia Skoura[2], Symeon Metallidis[1]

[1]Infectious Diseases Unit, 1st Internal Medicine Department, AHEPA University Hospital, School of Medicine, Aristotle University of Thessaloniki, Greece
Emails: olgatsachouridou.iasis@gmail.com (O.T.); pilalas_jim@hotmail.com (D.P.); sidnanoudis@yahoo.gr(S.N); 9anos95@gmail.com (A.A.); isidorabak@gmail.com (I.B.); teochriss@yahoo.co.uk (T.C.); conmark@windoslive.com (K.M); metallidissimeon@yahoo.gr (S.M.)
[2]Department of Microbiology, AHEPA University Hospital, School of Medicine, Aristotle University of Thessaloniki, Greece
Emails: angelikikasso@gmail.com (A.K.); vimantzana@gmail.com (P.M); protonotariou@auth.gr (E.P.); mollyskoura@gmail.com (L.S.)
* Correspondence: olgatsachouridou.iasis@gmail.com; Tel.: +302311388355;

Abstract: The incidence of multidrug-resistant (MDR) bloodstream infections (BSI) is associated with high morbidity and mortality. Little evidence exists regarding the epidemiology of BSIs and the use of appropriate empirical antimicrobial therapy in endemic regions. Novel diagnostic tests (RDTs) may facilitate and improve patient management. Data from patients with MDR GNB bacteremia at a university tertiary hospital were assessed over a 12-month period. 157 episodes of MDR GNB BSI were included in the study. Overall mortality rate was 50,3 percent. Rapid molecular diagnostic tests were used in 94% of BSI episodes. In univariate analysis, age (OR 1.05 (95% CI 1.03, 1.08) p<0.001), Charlson Comorbidity Index (OR 1.51 (95% CI 1.25, 1.83) p<0.001), Procalcitonin≥1(OR 3.67 (CI 95% 1.73, 7.79) p<0.001) and monotherapy with tigecycline (OR 3.64 (95% CI 1.13, 11.73) p=0.030) were the only factors associated with increased overall mortality. Surprisingly, time to appropriate antimicrobial treatment had no impact on mortality. MDR pathogen isolation, other than Klebsiella pneumoniae and Acinetobacter baumanii was associated with decreased mortality (OR 0.35 (95% CI 0.16, 0.79) p=0.011). In multivariate analysis though the only significant factor for mortality was Procalcitonin≥1(OR 2.84 (95% CI 1.13, 7.11) p=0.025). In conclusion, in an endemic area, mortality rates in MDR BSI remain high. High procalcitonin was the only variable that predicted death. The use of rapid diagnostics did not improve mortality rate.

Keywords: Gram-negative bacilli bacteremia; multidrug resistance; 28-day mortality; procalcitonin; rapid molecular diagnostics

Introduction

Blood stream infections (BSI) caused by multi-drug (MDR) Gram-negative bacteria (GNB) are a healthcare-associated issue causing the highest burden in quality of life and also associated with poor clinical outcomes [1,2].

They are relatively frequent among intensive care unit (ICU) patients and are associated with significant mortality [3–6]. Recently, worrisome increases in antimicrobial resistance have been highlighted globally[7-9]. Indeed, antimicrobial resistance is associated with delays to adequate antimicrobial therapy and increased mortality[10-11]. It leads to exacerbation in the use of broad-spectrum antimicrobial agents which in turn enhances the rise of more resistant pathogens [12-13]. The presence and spread of MDR GNB dramatically restricts treatment options for such nosocomial infections[14], whereas the pipeline of novel antimicrobial agents is slow and compounds including tigecycline, do not

© 2023 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2023 doi:10.20944/preprints202305.0858.v1

always prove to be promising[15-16]. Monotherapy with formerly abandoned antibiotics, such as polymyxins is another option [17]. However, apart from resistance, toxicity issues may arise [18].

Over the past years, Acinetobacter baumannii and Klebsiella pneumoniae have emerged as severe nosocomial pathogens due to their extensively resistant antimicrobial profile in blood cultures in endemic regions of Europe [19-21]. The extent of resistance may vary for each isolate; therefore, distinct definitions have been formatted. Bacteria expressing in vitro resistance to three or more antimicrobial categories are referred to as multidrug-resistant organisms [22].

The combined use of antimicrobial agents has been used in the management of infectious diseases, garnering more attention by clinicians lately due to the aforementioned reasons in Europe [23-24], but more importantly in the case of MDR Gram-negatives, it is expected to provide a probable antimicrobial synergy [25] and impove survival [26]. However,still empirical treatment remains inadequate in a significant proportion in Greece and mortality remains high in both ICU and medical wards setting[27]. Moreover, the local epidemiology and the limited feasibility for isolation of patients with MDR infections in Greek hospitals have an negative impact on efforts for effective infection control programs [21].

Although BSI occur less often than other nosocomial infections [28], the isolation of a pathogen in blood is solid evidence of severe infection [29]. Moreover, during the COVID-19 pandemic substantial rise of hospital onset of MDR infections in some Greek regions [30], compared to previous years. Though, this observation could be attributed to multiple reasons such as, prolonged length of stay of these patients, burst in the numbers of critically ill patients, the use of external devices and the excessive use of in-hospital antimicrobials.

Consequently, treatment of MDR-GNB remains a challenge. Since an effective treatment should be administered promptly, antimicrobial resistance almost invariably leads to inadequate empirical treatment, with possible negative consequences [31]. To optimize antimicrobial treatment apart form the knowledge of local epidemiology, medical history of patients and the risk of MDR GNB, the improvement and implementation of rapid molecular resistance typing techniques will assist the selection of the proper antibiotic. Finally, a rapid diagnosis will improve targeted therapy through prompt initiation of adequate therapy and de-escalation of antimicrobials when results are available [31].

Aim of this study was to investigate the impact of GNB BSI on the primary outcome of 28-day mortality of all causes. In addition, we tried to assess other risk factors for mortality in patients with GNB in an endemic area to both settings of ICU and medical wards during the last year of the COVID-19 pandemic.

2. Materials and Methods

2.1. Study Design, Setting and Selection criteria

This was a retrospective study from 1st of January 2022 until 31st of January 2023, that was carried out at AHEPA University Hospital, a 700-bed institution with four ICUs, as well as surgical and internal medicine departments, that serve patients from a large region in Northern Greece. Microbiology data were retrieved from the laboratory database, while medical history, epidemiological and treatment data were reviewed from the patients' electronic health records. The institutional medical scientific board approved this study (Scientific Council, Institutional Review Board AHEPA University Hospital, Ref. 018/ 06.02.2023)

The patients included in the study fulfilled the following criteria: adult ≥ 18 years old and first BSI episode due to a MDR GNB pathogen. We excluded individuals with Gram-positive, Gram-negative other than MDR or fungal BSI. Polymicrobial infections, defined as more than one pathogen isolated in a set of blood cultures, were also were included in the study even though such results may have influenced the provider's decision to

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2023 doi:10.20944/preprints202305.0858.v1

antimicrobial modification. Patients who died or were discharged prior to culture results were also excluded from the analysis.

No informed consent was required, as we handled the individual patient data anonymously. We report our results based on the Statement on Strengthening the Reporting of Observational Studies in Epidemiology [32].

2.2. Variables

Variables of interest on admission were: age, sex, Charlson Comorbidity Index [33], ICU admission during hospitalization, length of in-hospital stay, medical or surgical reason of hospitalization, infectious disease status, presence of immunosuppression or COVID-19 co-infection. We documented the day of the BSI event, its timing (<48h or ≥48 h from admission), the source of bacteremia, the type of pathogen isolated and whether the infection was monomicrobial of multi-microbial. We also recorded baseline creatinine and procalcitonin at the sample date of blood culture, antimicrobial resistance patterns in GNB blood culture isolates, use of rapid molecular diagnostic test (BIO-FIRE,Filmarray^{OR}) which was currently available in our hospital, antimicrobials used to treat the infection (empiric and targeted), appropriateness of empiric antibiotics as well as source control, time to targeted treatment, all-cause mortality within 28 days pf BSI episode, as long as actions taken upon receipt of microbiological results (antibiotic de-escalation, adequate targeted treatment).

2.3. Definitions

We defined MDR GNB-BSI as a positive blood culture for a MDR GNB, and the patient presented clinical and laboratory indices of infection. Index day was the date of collecting the first positive blood culture (index culture) that recovered a GNB isolate. We examined all patients' relevant MRD GNB BSI episodes for the purposes of the study. Bacteremias were assessed as primary or secondary based on whether the blood infection occurred directly or spread from another site of the body. Bacterial identification and antimicrobial susceptibility testing were performed using the automated system VITEK2 (bioMérieux, Marcy l'Etoile, France). Susceptibility testing results were interpreted according to the EUCAST breakpoints v 12.0 (2022) [34]. All isolates were tested phenotypically for the detection of MBL, KPC, or both categories using the meropenem disc test.

Further assessment was the modification of therapy within 24 hours of the reported susceptibilities. Modification was defined as either escalation of therapy to broader coverage or a de-escalation to a targeted agent based on the results of rapid diagnostic tests. Antibiotic therapy was categorized on empirical and targeted treatment, either as monotherapy either as combination of antimicrobials. Adequate empirical antibiotic therapy was defined as initiation of at least one antimicrobial agent to which the isolate from blood culture was susceptible within 24 hours after the blood sample drawn, definition adapted from previous studies [35].

Of note, many patients were not initially on broad-spectrum antimicrobials and in many others targeted therapy did not include a narrow-spectrum antibiotic. All outcomes measuring time were measured from the time of blood-culture draw based on results reported by the microbiology department. Other variables included time to targeted therapy, modification within 24 hours of susceptibility results, modification of treatment from empiric combination, hospital length of stay and mortality on day 28. Time to targeted therapy was defined as the time from culture drawn to the time of escalation or de-escalation to an antibiotic with in vitro activity against the isolated pathogen.

2.4. Outcome

The main study outcome was overall 28-day all-cause mortality after the MDR GNB BSI event. Secondary analysis was separately performed based on the isolate of BSIs with regards to mortality.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2023 doi:10.20944/preprints202305.0858.v1

2.5. Statistical analysis

Continuous variables were presented with mean and standard deviation or median and interquartile range (IQR), whereas categorical variables with frequencies and percentages. Logistic regression analysis was used to examine the association of different parameters with 28-day mortality. Parameters with a p-value < 0.2 in the univariate regression model as well as clinically significant parameters were entered into a multivariable regression model. The same analysis was run separately in patients with Acinetobacter baumanii and in patients with Klebsiella pneumoniae. Statistical analysis was performed using STATA 17.0 software and the significance level was set at α=0.05. We performed multivariable logistic regression analysis to evaluate risk factors for mortality in patients with MDR BSI.

3. Results

3.1. Baseline characteristics of patients with MDR GNB BSI episodes

A total of 157 MDR GNB BSI episodes were included in the study. Demographical, clinical and laboratory data are summarized in Table 1. Eighty-three patients were males (52.8%) and the mean age of the cohort was 67.63 years old. Nearly 58 percent of the episodes referred to patients from medical wards and 42% to ICU patients. The vast majority of the participants had a normal renal function upon admission, a mean Charlson Comorbidity Index below 4 and median length of hospital stay of 30.5 days. In fifty-six percent of episodes patient had a procalcitonin measure ≥ 1 ng/ml. Notably, forty-seven percent of patients never received an adequate antimicrobial therapy, while thirty-one percent received adequate treatment within 24 hours of the blood sample drawn. Nearly half of the isolates referred to infection from Acinetobacter baumanii, followed by Klebsiella pneumoniae (25%) and 25% other MDR GNB (Pseudomonas aeruginosa, Proteus mirabilis, Enterobacter cloacae, Providencia stuartii). Approximately, sixty percent of BSI episodes were primary bacteremias and 85,7% were monomicrobial. Rapid Diagnostic tests were used in the vast majority of cases (152/157). Empirical treatment included at least one active agent against GNB in less than one third of the episodes (31,06%), while 68% had no active antimicrobial in their initial empirical combination. The proportion of adequate treatment improved to 50,3% upon susceptibility results. Intervention with regards to discontinuation of unnecessary antimicrobial agents was reported in 36% of cases, while 68% of patients continued to receive an extended combination.

Thirty-four percent of MDR GNB bacteremias were reported to COVID-19 patients who were currently hospitalized during the study period, which highlights the impact of the pandemic on nosocomial morbidity even at its ending period.

MBL production was detected with different methods in 51 cases (31.68%), while KPC was reported in 37 cases (23%). Colistin was part of the empirical treatment in 14% of BSI episodes, tigecycline in 11%, and combination of both agents in 11% as well. Tigecycline was also part of combined empirical treatment with other novel antimicrobials such as ceftazidime/avibactam in lower rates (Table 1).

Table 1. Baseline characteristics of patients with MDR GNB bloodstream infections.

Variable	Total (N=157)	28-days mortality	
		Alive (N=78)	Dead (N=79)
Age, mean (sd)	67.63 (14.14)	62.98 (14.33)	72.28 (12.40)
Ward, N (%)			
ICU	66 (42.04)	29 (37.18)	37 (46.84)
Other	91 (57.96)	49 (62.82)	42 (53.16)
Creatinine Baseline, N (%)			
<1.2 mg/dl	123 (78.21)	65 (84.42)	57 (72.15)
1.2 – 1.9 mg/dl	25 (16.03)	8 (10.83)	17 (21.52)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2023 doi:10.20944/preprints202305.0858.v1

2 – 3.4 mg/dl	6 (3.85)	3 (3.85)	3 (3.38)
3.5 – 4.9 mg/dl	-	-	-
≥5 mg/dl	3 (1.92)	1 (1.30)	2 (2.53)
Charlson Comorbidity Index mean (sd)	3.89 (2.02)	3.17 (1.82)	4. 61 (1.96)
ICU days, median (IQR)	1 (0, 30)	3 (0, 45)	1 (0, 18)
Hospital days, median (IQR)	30.5 (19, 55)	47 (25, 77)	24 (16, 38)
PCT, N (%)			
< 1	54 (44.26)	36 (46.15)	18 (22.78)
≥ 1	68 (55.74)	24 (30.77)	44 (55.70)
Time to adequate antimicrobial therapy			
≤24 h	49 (31.41)	29 (37.18)	20 (25.36)
>24 h	33 (21.15)	17 (21.79)	16 (20.25)
None	74 (47.44)	32 (41.03)	42 (53.16)
GNB, N (%)			
Acinetobacter baumanii	75 (49.02)	31 (39.74)	44 (55.70)
Klebsiella pneumoniae	39 (25.49)	19 (24.36)	20 (25.32)
Other species	39 (25.49)	26 (33.33)	13 (16.46)
Type of Bacteremia, N(%)			
Primary	93 (59.24)	48 (61.54)	45 (56.96)
Secondary	64 (40.76)	30 (38.46)	34 (43.04)
Number of pathogens Isolated, N(%)			
1	135 (86.54)	68 (87.18)	67 (84.81)
≥ 2	21 (13.46)	10 (12.82)	11 (13.92)
Filmarray use, N (%)			
No	9 (5.73)	3 (3.85)	6 (7.59)
Yes	148 (94.27)	75 (96.15)	73 (92.41)
Active antibiotics in empirical treatment, N (%)			
0	108 (69.23)	49 (62.82)	59 (74.68)
≥ 1	48 (30.77)	29 (37.18)	19 (24.05)
Active antibiotics in targeted treatment, N (%)			
0	78 (50.00)	36 (46.15)	42 (53.16)
≥ 1	78 (50.00)	42 (53.85)	36 (45.56)
Intervention: Discontinuation of additional antibiotic, N (%)			
No	99 (63.46)	54 (69.23)	45 (56.96)
Yes	57 (36.54)	24 (30.77)	33 (41.77)
COVID-19 co-infection, N (%)			
No	104 (66.24)	50 (64.10)	54 (68.35)
Yes	53 (33.76)	28 (35.90)	25 (31.65)
MBL production	49 (31.21)	25 (32.05)	24 (30.38)
KPC production	36 (22.93)	15 (19.23)	21 (26.58)
Empirical: Colistin	22 (14.01)	12 (15.38)	10 (12.66)
Empirical: Tigecycline	17 (10.83)	4 (5.13)	13 (16.46)
Empirical:			

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2023 doi:10.20944/preprints202305.0858.v1

CAZ/AVI or MER/VAR	1 (0.64)	0 (0.00)	1 (1.27)
Empirical: Col + Tig	18 (11.46)	13 (16.67)	5 (6.33)
Empirical: Tig + CAZ/AVI	1 (0.64)	0 (0.00)	1 (1.27)
Empical: Tig + Col + CAZ/AVI	2 (1.27)	2 (2.56)	0 (0.00)

ICU: Intensive Care Unit; PCT: Procalcitonin; MBL: Metallo-β-Lactamases; KPC: Klebsiella producing Carbapenemases;Tig: Tigecycline; Col: Colistin; CAZ/AVI: Ceftazidime/ Avibactam; MER/VAR: Meropenem/Varbobactam.

Overall mortality reached 50.3% in this MDR GNB BSI cohort during the study period. More ICU patients with MDR GNB died (37 vs 29) in 28 days. This may be attributed to the fact that patients in the ICU are critically ill, more frequently septic and could inextricably die regardless of antimicrobial treatment. Patients who died had also higher CCI, higher procalcitonin count, more impaired renal function at admission and received less frequently adequate empirical or targeted treatment compared to those who survived until day 28.

3.2. Univariate and multivariate analysis for 28-day mortality

In univariate analysis for all-cause 28-day mortality (Table 2),we observed that for each one-year increase in age, the odds of death increased by 5% (OR 1.05 (95% CI 1.03, 1.08) p<0.001). More significantly, the odds of death increased by 51% for each one-unit increase in Charlson Comorbidity Index (OR 1.51 (95% CI 1.25, 1.83) p<0.001). The hospitalization setting (internal medicine ward, surgical ward, ICU) did not seem to affect the primary outcome of interest. Mortality from MRD GNB bacteremia was not also associated with the days of hospitalization even in the ICU, finding which is quite concerning for patients with less severe morbidity. The use of molecular rapid diagnostic tests was quite frequent in our cohort. However, this tool did not offer any positive impact to mortality in MDR GNB in an endemic environment, which highlights the need for further investigation on factors which will improve survival and in-hospital mordibidy in these patients.

Table 2. Univariate analysis for 28-day mortality.

Variable	**28-days mortality**	
	OR (95%)	**p-value**
Age	1.05 (1.03, 1.08)	<0.001*
Ward		
ICU	Ref.	
Other	0.67 (0.36,1.27)	0.221
Creatinine Baseline		
<1.2 mg/dl	Ref.	
1.2 – 1.9 mg/dl	2.42 (0.97, 6.05)	0.057
2 – 3.4 mg/dl	1.14 (0.22, 5.87)	0.875
3.5 – 4.9 mg/dl	-	-
≥5 mg/dl	2.28 (0.20, 25.82)	0.505
Charlson Comorbidity Index	1.51 (1.25, 1.83)	<0.001*
ICU days	0.98 (0.97, 0.99)	0.016*
Hospital days	0.97 (0.95, 0.98)	<0.001*
PCT		
< 1	Ref.	
≥ 1	3.67 (1.73, 7.79)	0.001*
Time to adequate antimicrobial therapy		
≤24 h		

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2023 doi:10.20944/preprints202305.0858.v1

>24 h	Ref.	
None	1.36 (0.56, 3.32)	0.493
	1.90 (0.92, 3.95)	0.085
GNB		
Acinetobacter	Ref.	
Klebsiella	0.74 (0.34, 1.64)	0.452
Other	0.35 (0.16, 0.79)	0.011*
Type of Bacteremia		
Primary	Ref.	
Secondary	1.21 (0.64, 2.29)	0.560
Number of pathogens Isolated		
1	Ref.	
≥ 2	1.12 (0.44, 2.80)	0.815
Filmarray use (Y/N)		
No	Ref.	
Yes	0.49 (0.12, 2.02)	0.321
Active antibiotics in empirical treatment, N (%)		
0	Ref.	
≥ 1	0.54 (0.27, 1.09)	0.085
Active antibiotics in targeted treatment, N (%)		
0	Ref.	
≥ 1	0.73 (0.39, 1.38)	0.337
Intervention: Discontinuation of additional antibiotic, N (%)		
No	Ref.	
Yes	1.65 (0.85, 3.19)	0.136
COVID COVID-19 co-infection, N (%)		
No	Ref.	
Yes	0.83 (0.74, 1.59)	0.573
MBL production		
No	Ref.	
Yes	0.93 (0.47, 1.81)	0.821
KPC production		
No	Ref.	
Yes	1.52 (0.72, 3.23)	0.275
Empirical treatment :Colistin		
No	Ref.	
Yes	0.79 (0.32, 1.97)	0.623
Empirical treatment: Tigecycline		
No	Ref.	
Yes	3.64 (1.13, 11.73)	0.030*
Empirical treatment: Col + tig		
No	Ref.	
Yes	0.34 (0.11, 1.00)	0.050
OR: Odds Ratio, CI: confidence interval		
*Statistically significant at level 0.05		

ICU: Intensive Care Unit; PCT: Procalcitonin; GNB: Gram negative bacteria;MBL: Metallo-β-Lactamases; KPC: Klebsiella producing Carbapenemases; Col: Colistin Tig: Tigecycline.

Monomicrobial infections were not associated with lower mortality rates from MDR Gram-negative bacteria, which is also an important finding. Our cohort included a non-neglectable proportion of multi-microbial bacteremias reaching 13% of the total episodes,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2023 doi:10.20944/preprints202305.0858.v1

during the study period of interest. Literature remains ambiguous concerning polymicrobial vs monomicrobial multi-drug gram negative bacteremias.

Patients with PCT≥ 1 ng/ml at the time of blood sample drawn had 3.7 times higher odds of death than the patients with PCT<1 ng/ml OR 3.67 (CI 95% 1.73, 7.79) p<0.001), which firmly supports the use of procalcitonin in severely affected patients. Patients with BSI isolates other than Acinetobacter baumanii or Klebsiella pneumoniae had 65% lower odds of death compared to patients with Klebsiella pneumoniae (OR 0.35 (95% CI 0.16, 0.79) p=0.011). Administration of tigecycline as empiric monotherapy in an endemic area showed to have a negative impact on patients, since they had 3.6 times higher odds of death compared to those who didn't (OR 3.64 (95% CI 1.13, 11.73) p=0.030). Patients with ≥ 2 active antibiotic agents in targeted treatment had 65% lower odds of death compared to patients with no adequate targeted treatment (Table 2).

Isolation of pathogens with different mechanisms of antimicrobial resistance didn't display a role to 28-day mortality among bacteremic patients with multi-drug resistant gram-negative bacteria. This finding could probably be explained in the setting of endemicity of MDR Gram-negative pathogens in blood of hospitalized patients. Overall mortality remains high, and more prevention measures and treatment management protocols need to be evaluated and implemented to improve outcomes.

Other probable risk factors for the outcome of interest were the number of active drug agents in both empirical and targeted treatment. Even though it would be expected that more effective antimicrobials in a prescribed regimen would be life-saving for hospitalized patients, our data couldn't confirm an association with lower 28-day mortality. Furthermore, modification of treatment post susceptibility results and discontinuation of unnecessary agents had no impact on the outcome. Continuation of administration of redundant agents may be associated with toxicity and adverse events, like Clostridioides difficile colitis, thus increasing the risk for morbidity and mortality, but it wasn't illustrated here.

In multivariate analysis (Table 3), only patients with procalcitonin count ≥1 had 2.8 times higher odds of death than patients with procalcitonin <1, adjusted for all the other variables in the model (OR 2.84 (95% CI 1.13, 7.11) p=0.025).

Table 3. Multivariable analysis for 28-day mortality.

Variable	28-days mortality			
	Univariate analysis		Multivariable analysis	
	OR (95%)	p-value	OR (95%)	p-value
Age	1.05 (1.03, 1.08)	<0.001*	1.03 (0.98, 1.08)	0.234
Ward				
ICU	Ref.	0.221	Ref.	0.218
Other	0.67 (0.36,1.27)		0.54 (0.21, 1.42)	
Charlson Comorbidity Index	1.51 (1.25, 1.83)	<0.001*	1.25 (0.88, 1.77)	0.213
PCT				
< 1	Ref.	0.001*	Ref.	0.025*
≥ 1	3.67 (1.73, 7.79)		2.84 (1.13, 7.11)	
Time to adequate antimicrobial therapy				
≤24 h	Ref.		Ref.	
>24 h	1.36 (0.56, 3.32)	0.493	1.36 (0.40, 4.61)	0.616
None	1.90 (0.92, 3.95)	0.085	1.45 (0.50, 4.19)	0.494
GNB				
Acinetobacter baumanii	Ref.		Ref.	
Klebsiella pneumoniae	0.74 (0.34, 1.64)	0.452	0.78 (0.29, 2.12)	0.635
Other species	0.35 (0.16, 0.79)	0.011*	0.74 (0.24, 2.23)	0.588

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2023 doi:10.20944/preprints202305.0858.v1

Empirical treatment Tigecycline				
No	Ref.		Ref.	
Yes	3.64 (1.13, 11.73)	0.030*	3.66 (0.64, 21.08)	0.146
Empirical treatment Col + tig				
No	Ref.		Ref.	
Yes	0.34 (0.11, 1.00)	0.050	0.64 (0.15, 2.74)	0.544
OR: Odds Ratio, CI: confidence interval				
*Statistically significant at level 0.05				

ICU: Intensive Care Unit; PCT: Procalcitonin; GNB: Gram Negative Bacteria; Tig: Tigecycline; Col: Colistin.

3.3. Patients with BSI from MDR Acinetobacter baumanii.

When we performed univariate analysis among patients with MDR Acinetobacter baumanii bacteremia, we observed that for each one-year increase in age, the odds of death increase by 9% (OR 1.09 (95% CI 1.04, 1.14)p <0.001). With regards to CCI, for each one-unit increase in Charlson Comorbidity Index, the odds of death increase by 78% (OR 1.78 (95% CI 1.29, 2.46) p <0.001). Similarly with the whole cohort of BSIs, patients with PCT≥ 1 have 3.5 times higher odds for mortality than the patients with PCT<1 (OR 3.45 (95% CI 1.08, 1.03) p= 0.037). Co-administration of colistin plus tigecycline in empirical treatment led patients to decreased odds of death by 79% (OR 0.21 (0.05, 0.87) p=0.032) [data not shown]

3.4. Patients with BSI from MDR Klebsiella pneumoniae

Accordingly, when studying separately patients with Klebsiella pneumoniae using univariate analysis, we observed that having PCT≥ 1 have 3.7 times higher odds of death than the patients with PCT<1. Additionally, Patients with more than 1 active antibiotic in empirical treatment have 84% lower odds of death compared to patients with no active antibiotic agents in their initial antibiotic regimen. As could have been expected, patients with time to adequate antimicrobial therapy greater than 24h were assessed to have 7.5 higher odds of death than those with time to adequate antimicrobial therapy lower than 24h, highlighting the fundamental principal of infection control that time to appropriate treatment is of great significance for an optimal outcome [data not shown].

4. Discussion

The prevalence of bloodstream infections attributed to MDR GNB is currently rising with negative impact on morbidity and mortality. Epidemiology data of prevalence and circulating antimicrobial resistance patterns of GNB BSI isolates from hospitalized patients, as well as identification of risk factors for harboring MDR GNB infections, may facilitate patient care [36]. Our aim was to describe mortality in bacteremic patients from MDR Gram-negative bacteria in a tertiary hospital in Thessaloniki Greece, a region endemic for multi-drug and difficult to treat Gram-negative hospital infections.

Our study revealed a rather high case mortality rate of 50,3% among patients with MDR GNB bloodstream infections, much higher than previous published studies [37] of hospitalized patients. Mortality rate was assessed lower (41,6%) even in neutropenic patients [38], pediatric (21,4%) population [39] or ICU (45%) patients [40,41]. Our population differs from the above in terms of heterogeneity, with regards to medical history, comorbidity status and hospital ward origin (both ICU and non- ICU participants). This could have accounted for such differences in mortality rates. Several studies pointed out that ICU admission is a risk factor for worst survival [42], finding thus not confirmed in our data, reporting high mortality rates in both hospitalization settings. However, we should mention that in non-endemic countries for MDR GNB bacteria, mortality rates have reported even more dramatic in the ICU setting [43]. However, we should underline that

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2023 doi:10.20944/preprints202305.0858.v1

ICU patients are critically ill and may suffer from sepsis, thus inevitably die irrespectively of antimicrobial treatment.

Patients with MDR GNB infections are more likely to receive inadequate empirical treatment [44] leading to poorer outcomes, such as increased mortality and prolonged hospitalization [45]. In a large multicenter study of ICU patients run in 52 countries, adequate antimicrobial therapy was received by 51.5% within 24 h of blood culture drawn. Additionally, antimicrobial resistance was associated with delay to adequate antimicrobial treatment [41]. In our study, inadequate empirical treatment was not associated with higher mortality, in contrast with previous studies [37, 46]. This could be probably attributed to large proportions of inadequate both empirical and targeted treatment options in this study, along with being investigated in an endemic setting for MDR GNB.

Ten years after a similar multicenter cohort study critically ill patients with BSIs [3], comparable delay to adequate antimicrobial therapy were reported by others [41], underlining the need for implementation of integrated protocols and infection control programs to better predict antimicrobial resistance and source control. Antimicrobial resistance was associated with delay in administration of effective treatment. Delayed adequate antimicrobial therapy was not associated with day-28 mortality. Such observation may be impacted by several confounders and should be interpreted with caution, also underlined by other larger studies [41]. Limited data reported adequate antibiotic therapy in ICU patients with MDR bacteria. An Italian study reported 61% inadequate treatment for MDR infections [47], which is more consistent with our rates, in both hospital settings however.

Indeed, many observational studies consider the possible relation between all-cause mortality and time to appropriate antimicrobial therapy as complicated and difficult to be clarified [48]. On the one hand, the clinical assessment of severity of infection may guide prompt administration of broad-spectrum antimicrobials to patients at higher risk of death and thus, to confound the results of such studies.

Findings of this study do not relegate early adequate antimicrobial therapy recommendation for patients with severe infections. Indeed, while avoidance of antibiotic overuse and its associated adverse events [49], primary adequate antimicrobial treatment remains an intervention of great significance for nosocomial BSIs [50]. Integrated infection control and antibiotic stewardship programs may facilitate clinical management providing advice and recommendations on antibiotic selection, mode and dosing of administration, as well as schedule for monitoring clinical and laboratory course of treatment [50].

Comorbidities have been assessed to play a significant role in worse outcomes [37-38] and high mortality rates. In our study higher Charlson Comorbidity Index was risk factor for high mortality in univariate analysis, which is in line with other authors [37, 42]. Remarkably, CCI score >3 was also associated with more frequently administration of inadequate empirical treatment, according to previous authors [44]. Blot et al. reported that bacteremia of MDR Pseudomonas spp was a risk factor for mortality [40]. This finding is in contrast with our results, in which isolation of other than Acinetobacter baumanii and Klebsiella pneumoniae species was associated with better survival.

One third of MDR GNB BSIs of our cohort were reported among COVID-19 patients who were currently hospitalized during the study period, which highlights the significance of severe secondary infections in co-infected patients during the remission phase of pandemic. Indeed, COVID-19 infection modified incidence and severity of nosocomial infections in several countries. [51]. In recently published data from our hospital, an increase of secondary BSIs among COVID-19 patients [30], which was in lineage with other reports [52]. In 2020, we observed a notable increase of BSIs presented with more resistant phenotypes of the isolates when compared with the respective rates before the onset of the pandemic. A remarkable increase (almost 50 percent) of K. pneumoniae carbapenem resistance rates was observed between 2019 and 2020. Resistance to colistin also increased for A. baumannii, K. pneumoniae and P. aeruginosa, the three more endemic species in Greek hospitals. Notably, the incidence of BSIs in COVID-19 patients in our hospital during the second epidemic wave, was one of the highest published in the literature, whereas the more prevalent causative pathogens were MDR Gram-negative [30]. This observation

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2023 doi:10.20944/preprints202305.0858.v1

could be possibly explained by their prolongation of hospitalization and the extensive antimicrobial regime that these patients received. However, COVID-19 co-infection was not a risk factor for mortality in our study during the remission phase of the pandemic as reported here.

Furthermore, we studied whether time for treatment modification played a role in mortality, as well as we assessed actions like discontinuation of redundant antibiotic agent and if the number of active agents of both empirical and targeted treatment was associated with the main outcome of death on day 28. Even though it would be expected that more effective antimicrobials in a prescribed regimen would be life-saving for hospitalized patients, our data couldn't confirm an association with lower 28-day mortality. Additionally, modification of treatment post susceptibility results receipt and discontinuation of unnecessary agents had no impact on the main outcome of interest.

Previous studies reported that inadequate treatment is associated with higher odds of negative outcomes [53]. In grounds of widespread resistance to broad-spectrum antibiotic agents, implementation of molecular rapid diagnostic tests may be a key for prompt adequate antimicrobial therapy [54-55]. In this study we also evaluated the possible effect of using RDT on mortality in patients with MDR GNB bacteremia. Mohayya et al showed recently that reduction in duration of inadequate empirical treatment was associated with better outcomes and despite not being statistically significant, the finding was notable and may favor the use of RDT as a useful tool for adequate targeted treatment in the context of antimicrobial resistance strategies [56]. Although the implementation of antibiotic stewardship protocols and the progress and handy release of diagnostic tools might optimize appropriate empirical therapy, selecting appropriate empirical therapy remains a challenge, particularly for resistant pathogens. Recently published data from US of a large cohort display a positive effect of appropriate empirical treatment on mortality during hospitalization [57].

More recent studies assessed the impact of rapid diagnostics in outcomes of patients with MDR infections [58-59]. These studies demonstrated an improvement in administration of prompt adequate treatment using RDTs, findings consistent with our data which report an improvement in time of first modification. Other authors, reported improved exploitation of antibiotics for gram-negative bacteria in critically ill patients. Our data add to the literature by expanding these findings to all patients with MDR gram-negative bacteremia, not just ICU patients, suggesting a broader real-world encounter. Furthermore, our study was conducted during the remission year of COVID-19 pandemic, so the texture of the cohort was miscellaneous regarding comorbidities and disease severity.

Advances in diagnostic approaches, as well as implementation of antimicrobial stewardship programs, may play an important role in ensuring that patients receive adequate treatment in a timelier fashion than in the past [60-62]. However, data are conflicting regarding the impact of RDT use on clinical outcomes with either optimization or no impact on clinical outcomes [59, 63-65]. Babowicz et al. suggested improvement in mortality rates in contrast with our results and previous large studies [63]. In this study, the use of molecular rapid diagnostic tests did not seem to have a positive effect on reduction of the mortality rate among patients with MDR GNB bloodstream infections. Despite not improving the outcome of interest, rapid modification and augmentation of adequate treatment rates may potentially upgrade long-term outcomes.

Despite the fact that concomitant isolation of GNB generally is not reported to affect mortality in patients with MDR Acinetobacter baumanii, it was associated with worst outcomes in general [66]. In this study, monomicrobial infections were not associated with lower mortality rates from all MDR Gram-negative bacteria, which is also an important finding and compatible with other authors. Although multiple studies have reported higher mortality rates in bacteremic patients with polymicrobial infection [67-68], the attributable mortality rate varied depending on the causative pathogen isolated [69-70]. Compared with monomicrobial bacteremia, polymicrobial bacteremia of P. aeruginosa [71] was associated with higher mortality, while polymicrobial Klebsiella pneumoniae bacteremia did not led to worse outcome [72]. These findings indicate that the influence

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2023 doi:10.20944/preprints202305.0858.v1

of polymicrobial bacteremia on prognosis should be assessed separately, like we tried to further highlight in this study.

Limitations

This study has few limitations. First of all, being a single-center study, the results might have been affected by the practices exclusive applied in this particular health-care facility, thereby limiting the generalizability of our findings. However, the medical wards and the ICU are part of the large tertiary-care hospital that serves patients from different regions of Northern Greece. Secondly, no antibiotic dosing data and modification of dosage administration were available in this study, so it is not fully confirmed that patients received optimal treatment, factor that might have an impact on outcome. This uncertainty might have affected the definition of adequate antibiotic therapy. Third, due to the study's retrospective design, may not account for all confounding factors. However, precise consideration was taken to minimize these factors. Nevertheless, we attempted to collect all study-related information for all patients. By including merely GNB BSI patients, we tried to mitigate the risk of including contaminated blood cultures, which did not require treatment. Additionally, MDR GNB infections remain a major issue in Greek hospitals. The strength of the study could be impacted by not assessing risk factors for the development of MDR GNB BSIs in order to suggest effective measures for prevention of difficult to treat nosocomial infections. Lastly, the small size of the cohort limited the ability to perform multivariate analysis separately for Acinetobacter baumanii and Klebsiella pneumoniae BSI cases, to further investigate impact of the responsive bacteria on mortality. Despite the limitations, the study has several strengths. The study focused on MDR GNB bloodstream infections on both wards and ICU and reported on important clinical outcomes, like mortality rate, which remains high in an endemic area, and still literature data is inconsistent. In addition, a detailed description on the use of antibiotics and actions taken within the first 24 h of susceptibility results release were also presented, in a setting where rapid diagnostic tests are in use for infection control purposes. The greatest strength of our study is its real-world impact assessment which might set a guide for improving clinical outcomes in patients with difficult to treat bacteremias and reinforce nosocomial infection prevention practices for clinical management.

5. Conclusions

In conclusion, this study aimed to assess clinical prognosis through 28-day all-cause mortality among hospitalized patients with bloodstream infections of multi-drug resistant Gram-negative bacteria. We report, a rather high mortality rate in our cohort which derives from an endemic region for MDR GNB. Severity of bacterial infections, indirectly assessed by higher PCT count, was an independent predictor of mortality, regardless other risk factors, which is consistent with previous studies and highlight its use in daily practice.

Several factors that could affect the outcome of interest were investigated in this study in both ICU and ward setting, wihtout nevertheless leading to conclusive results. Over all, our study has revealed high rates of MDR BSIs among the hospitalized COVID-19 patients, finding with significant implications for active surveillance and need for clinical management with the appropriate antibiotics for secondary infections even during a remission phase of the pandemic. Finally, the use of molecular rapid diagnostic tests did not seem to have a positive effect on reduction of the mortality rate among patients with MDR GNB bloodstream infections. A judicious selection of broad empirical antimicrobial regimen is essential, but a comprehensive approach would also be warranted to further improve outcomes. In summary, further prospective studies are needed to define optimal strategies for adequate empirical treatment and management in endemic for MDR Gram-negative bacteria regions.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2023 doi:10.20944/preprints202305.0858.v1

Author Contributions: O.T. contributed to supervision, interpretation and design, drafted and revised the manuscript. D.P. contributed to conception, supervision and designed the manuscript. S.N. contributed to data acquisition and interpretation and drafted the manuscript. A.A. contributed to design, data acquisition and data interpretation, and drafted the manuscript. I.B. contributed to data acquisition and interpretation. T.C. contributed to data acquisition. K.M. contributed to data acquisition. A.K. contributed to data acquisition. P.M. contributed to data acquisition. E.P. contributed to data acquisition and interpretation. L.S. contributed to data acquisition and interpretation. S.M. contributed to supervision and interpretation, and critically revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Vardakas, K.Z.; Rafailidis, P.I.; Konstantelias, A.A.; Falagas, M.E. Predictors of mortality in patients with infections due to multi-drug resistant Gram-negative bacteria: The study, the patient, the bug or the drug? J. Infect. 2013, 66, 401–414.
2. Lemos EV, de la Hoz FP, Alvis N, et al. Impact of carbapenem resistance on clinical and economic outcomes among patients with Acinetobacter baumannii infection in Colombia. *Clin Microbiol Infect*. 2014;20(2):174-180.
3. Tabah A, Koulenti D, Laupland K, et al. Characteristics and determinants of outcome of hospital-acquired bloodstream infections in intensive care units: the EUROBACT International Cohort Study. Intensive Care Med. 2012;38(12):1930-1945.
4. Vincent JL, Sakr Y, Singer M, et al. Prevalence and Outcomes of Infection Among Patients in Intensive Care Units in 2017. JAMA. 2020;323(15):1478-1487.
5. Adrie C, Garrouste-Orgeas M, Ibn Essaied W, et al. Attributable mortality of ICU-acquired bloodstream infections: Impact of the source, causative micro-organism, resistance profile and antimicrobial therapy. J Infect. 2017;74(2):131-141.
6. Prowle JR, Echeverri JE, Ligabo EV, et al. Acquired bloodstream infection in the intensive care unit: incidence and attributable mortality. Crit Care. 2011;15(2): R100.
7. De Waele JJ, Akova M, Antonelli M, et al. Antimicrobial resistance and antibiotic stewardship programs in the ICU: insistence and persistence in the fight against resistance. A position statement from ESICM/ESCMID/WAAAR round table on multi-drug resistance. Intensive Care Med. 2018;44(2):189-196.
8. World Health Organization (2021) Global antimicrobial resistance and use surveillance system (GLASS) report: 2021.
9. Bezabih YM, Bezabih A, Dion M, et al. Comparison of the global prevalence and trend of human intestinal carriage of ESBL-producing Escherichia coli between healthcare and community settings: a systematic review and meta-analysis. JAC Antimicrob Resist. 2022;4(3): dlac048.
10. Cosgrove SE. The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. Clin Infect Dis. 2006;42 Suppl 2:S82-S89.
11. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis [published correction appears in Lancet. 2022 Oct 1;400(10358):1102]. *Lancet*. 2022;399(10325):629-655.
12. Ríos, E.; Del Carmen López Diaz, M.; Culebras, E.; Rodríguez-Avial, I.; Rodríguez-Avial, C. Resistance to fosfomycin is increasing and is significantly associated with extended-spectrum β-lactamase-production in urinary isolates of Escherichia coli. Med. Microbiol. Immunol. 2022, 211, 269–272.
13. Meletis, G.; Skoura, L. Polymyxin resistance mechanisms: From intrinsic re-sistance to mcr genes. Recent Pat. Antiinfect. Drug Discov. 2018, 13, 198–206.
14. Meletis, G. Carbapenem resistance: Overview of the problem and future perspectives. Ther. Adv. Infect. Dis. 2015, 3, 15–21.
15. Seifert, H.; Blondeau, J.; Lucaßen, K.; Utt, E.A. Global update on the in vitro activity of tigecycline and comparators against isolates of Acinetobacter baumannii and rates of resistant phenotypes (2016–2018). J. Glob. Antimicrob. Resist. 2022, 31, 82–89.
16. Zak-Doron Y, Dishon Benattar Y, Pfeffer I, et al. The Association Between Empirical Antibiotic Treatment and Mortality in Severe Infections Caused by Carbapenem-resistant Gram-negative Bacteria: A Prospective Study. *Clin Infect Dis*. 2018;67(12):1815-1823.
17. Kadri SS, Adjemian J, Lai YL, et al. Difficult-to-Treat Resistance in Gram-negative Bacteremia at 173 US Hospitals: Retrospective Cohort Analysis of Prevalence, Predictors, and Outcome of Resistance to All First-line Agents. *Clin Infect Dis*. 2018;67(12):1803-1814.
18. Jafari, F.; Elyasi, S. Prevention of colistin induced nephrotoxicity: A review of preclinical and clinical data. Expert Rev. Clin. Pharmacol. 2021, 14, 1113–1131.
19. Meletis, G. Carbapenem resistance: Overview of the problem and future perspectives. Ther. Adv. Infect. Dis. 2016, 3, 15–21.
20. Mancuso, G.; Midiri, A.; Gerace, E.; Biondo, C. Bacterial antimicrobial resistance: The most critical pathogens. Pathogens 2021,10, 1310.
21. Protonotariou E, Meletis G, Pilalas D, et al. Polyclonal Endemicity of Carbapenemase-Producing Klebsiella pneumoniae in ICUs of a Greek Tertiary Care Hospital. Antibiotics (Basel). 2022;11(2):149.)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2023 doi:10.20944/preprints202305.0858.v1

22. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18:268–281.
23. Mantzana P, Protonotariou E, Kassomenaki A, et al. In Vitro Synergistic Activity of Antimicrobial Combinations against Carbapenem- and Colistin-Resistant Acinetobacter baumannii and Klebsiella pneumoniae. Antibiotics (Basel). 2023;12(1):93.
24. Papst L, Beović B, Pulcini C, et al. Antibiotic treatment of infections caused by carbapenem-resistant Gram-negative bacilli: an international ESCMID cross-sectional survey among infectious diseases specialists practicing in large hospitals. Clin Microbiol Infect. 2018;24(10):1070-1076.
25. Bergen, P.J.; Smith, N.M.; Bedard, T.B.; Bulman, Z.P.; Cha, R.; Tsuji, B.T. Rational com-binations of polymyxins with other antibiotics. Adv. Exp. Med. Biol. 2019, 1145, 251–288.
26. Vardakas, K.Z.; Athanassaki, F.; Pitiriga, V.; Falagas, M.E. Clinical relevance of in vitro synergistic activity of antibiotics for multidrug-resistant Gram-negative infections: A systematic review. J. Glob. An-timicrob. Resist. 2019, 17, 250–259.
27. Karvouniaris M, Poulakou G, Tsiakos K, et al. ICU-Associated Gram-Negative Bloodstream Infection: Risk Factors Affecting the Outcome Following the Emergence of Colistin-Resistant Isolates in a Regional Greek Hospital. Antibiotics (Basel). 2022;11(3):405.
28. Vincent JL, Rello J, Marshall J, et al. International study of the prevalence and outcomes of infection in intensive care units. *JAMA*. 2009;302(21):2323-2329.
29. Kalil AC, Metersky ML, Klompas M, et al. Management of Adults With Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clin Infect Dis. 2016;63(5):e61-e111.
30. Protonotariou E, Mantzana P, Meletis G, et al. Microbiological characteristics of bacteremias among COVID-19 hospitalized patients in a tertiary referral hospital in Northern Greece during the second epidemic wave. FEMS Microbes. 2021;2: xtab021.
31. Bassetti M, Kanj SS, Kiratisin P, et al. Early appropriate diagnostics and treatment of MDR Gram-negative infections. JAC Antimicrob Resist. 2022;4(5): dlac089.
32. Vandenbroucke, J.P.; von Elm, E.; Altman, D.G.; Gøtzsche, P.C.; Mulrow, C.D.; Pocock, S.J.; Poole, C.; Schlesselman, J.J.; Egger, M. For the STROBE Initiative Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and Elaboration. PLoS Med. 2007, 4, e296.
33. Charlson, M.E.; Pompei, P.; Ales, K.L.; MacKenzie, C.R. A new method of classifying prognostic comorbidity in longitudinal studies: Development and validation. J. Chronic Dis. 1987, 40, 373–383.
34. European Committee on Antimicrobial Susceptibility Testing. Breakpoint Tables for Interpretation of MICs and Zone Diameters Version 12.0. Available online: http://www.eucast.org (Accessed on 10 March 2023).
35. Micek ST, Welch EC, Khan J, et al. Empiric combination antibiotic therapy is associated with improved outcome against sepsis due to gram-negative bacteria: a retrospective analysis. Antimicrob Agents Chemother. 2010;54(5):1742–1748.
36. Pop-Vicas AE and D'Agata EM. The rising influx of multidrug-resistant Gram-negative bacilli into a tertiary care hospital. Clin Infect Dis 2005; 40: 1792–1798.
37. 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(1):11-18.
38. Gudiol C, Bodro M, Simonetti A, et al. Changing aetiology, clinical features, antimicrobial resistance, and outcomes of bloodstream infection in neutropenic cancer patients. J Antimicrob Chemother 2011; 66: 657–663.
39. Tsai MH, Chu SM, Hsu JF, et al. Risk factors and outcomes for multidrug-resistant Gram-negative bacteremia in the NICU. Pediatrics 2014; 133: e322–e329.
40. Blot S, Vandewoude K, De Bacquer D, et al. Nosocomial bacteremia caused by antibiotic-resistant Gram-negative bacteria in critically ill patients: clinical outcome and length of hospitalization. Clin Infect Dis 2002; 34: 1600–1606.
41. Tabah A, Buetti N, Staiquly Q, et al. Epidemiology and outcomes of hospital-acquired bloodstream infections in intensive care unit patients: the EUROBACT-2 international cohort study. *Intensive Care Med*. 2023;49(2):178-190.
42. Lye DC, Earnest A, Ling ML, et al. The impact of multidrug resistance in healthcare-associated and nosocomial Gram-negative bacteraemia on mortality and length of stay: cohort study. Clin Microbiol Infect 2012; 18: 502–508.
43. Alkofide H, Alhammad AM, Alruwaili A, et al. Multidrug-Resistant and Extensively Drug-Resistant Enterobacteriaceae: Prevalence, Treatments, and Outcomes - A Retrospective Cohort Study. Infect Drug Resist. 2020; 13:4653-4662.
44. Dietl B, Boix-Palop L, Gisbert L, et al. Risk factors associated with inappropriate empirical antimicrobial treatment in bloodstream infections. A cohort study. Front Pharmacol. 2023; 14:1132530.
45. MacVane SH, Tuttle LO and Nicolau DP. Impact of extended-spectrum β-lactamase-producing organisms on clinical and economic outcomes in patients with urinary tract infection. J Hosp Med 2014; 9: 232–238.
46. Rottier WC, Ammerlaan HS, Bonten MJ, et al. Effects of confounders and intermediates on the association of bacteraemia caused by extended-spectrum β-lactamase-producing Enterobacteriaceae and patient outcome: a metaanalysis. J Antimicrob Chemother 2007; 60: 913–920.
47. Viceconte G, Maraolo AE, Iula VD, Catania MR, Tosone G, Orlando R. Appropriateness of antibiotic prescription for targeted therapy of infections caused by multidrug-resistant bacteria: assessment of the most common improper uses in a tertiary hospital in southern Italy. Infez Med. 2017;25(3):224–233.
48. Weinberger J, Rhee C, Klompas M. A Critical Analysis of the Literature on Time-to-Antibiotics in Suspected Sepsis. J Infect Dis. 2020;222(Suppl 2): S110-S118.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2023 doi:10.20944/preprints202305.0858.v1

49. Curran J, Lo J, Leung V, et al. Estimating daily antibiotic harms: an umbrella review with individual study meta-analysis. Clin Microbiol Infect. 2022;28(4):479-490.
50. Evans L, Rhodes A, Alhazzani W, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. Intensive Care Med. 2021;47(11):1181-1247.
51. Buetti N, Tabah A, Loiodice A, et al. Different epidemiology of bloodstream infections in COVID-19 compared to non-COVID-19 critically ill patients: a descriptive analysis of the Eurobact II study. *Crit Care*. 2022;26(1):319.
52. Pasquini Z, Barocci I, Brescini L, et al. Bloodstream infections in the COVID-19 era: results from an Italian multi-centre study. Int J Infect Dis. 2021; 111:31-36.
53. Teshome BF, Vouri SM, Hampton N, Kollef MH, Micek ST. Duration of exposure to antipseudomonal β-lactam antibiotics in the critically ill and development of new resistance. Pharmacotherapy 2019;39:261–270.
54. Giacobbe DR, Giani T, Bassetti M, Marchese A, Viscoli C, Rossolini GM. Rapid microbiological tests for bloodstream infections due to multidrug resistant Gram-negative bacteria: therapeutic implications. Clin Microbiol Infect. 2020;26(6):713-722.
55. Banerjee R, Teng CB, Cunningham SA, et al. Randomized Trial of Rapid Multiplex Polymerase Chain Reaction-Based Blood Culture Identification and Susceptibility Testing. Clin Infect Dis. 2015;61(7):1071-1080.
56. Mohayya SM, Arsalan M, Narayanan N, et al. Impact of phenotypic rapid diagnostic assay on duration of empiric antibiotics for gram-negative bacteremia. Antimicrob Steward Healthc Epidemiol. 2023;3(1):e22.
57. Ohnuma T, Chihara S, Costin B, et al. Association of Appropriate Empirical Antimicrobial Therapy With In-Hospital Mortality in Patients With Bloodstream Infections in the US. JAMA Netw Open. 2023;6(1):e2249353.
58. MacVane SH, Bhalodi AA, Dare RK, et al. Improving outcomes and antibiotic stewardship (IOAS) for patients with gram-positive bloodstream infections through use of rapid testing: a quasi-experimental multicentre study of the Accelerate PhenoTest BC Kit. J Antimicrob Chemother 2021; 76:2453–2463.
59. Banerjee R, Komarow L, Virk A, et al. Randomized trial evaluating clinical impact of RAPid IDentification and susceptibility testing for gram-negative bacteremia: RAPIDS-GN. Clin Infect Dis 2020; 73:e39–e46.
60. Tsalik EL, Bonomo RA, Fowler VG Jr. New molecular diagnostic approaches to bacterial infections and antibacterial resistance. *Annu Rev Med*. 2018;69:379-394.
61. Timbrook TT, Morton JB, McConeghy KW, Caffrey AR, Mylonakis E, LaPlante KL. The effect of molecular rapid diagnostic testing on clinical outcomes in bloodstream infections: a systematic review and meta-analysis. *Clin Infect Dis*. 2017;64(1):15-23.
62. Akpan MR, Isemin NU, Udoh AE, Ashiru-Oredope D. Implementation of antimicrobial stewardship programmes in African countries: a systematic literature review. J Glob Antimicrob Resist. 2020;22:317-324.
63. Babowicz F, LaPlante R, Mitchell C, et al. Impact of Accelerate Pheno and BacT/Alert Virtuo on clinical processes and outcomes in patients with sepsis and concurrent gram-negative bacteremia. Antimicrob Agents Chemother 2021;65: e02364–20.
64. Dare RK, Lusardi K, Pearson C, et al. Clinical impact of Accelerate Pheno rapid blood culture detection system in bacteremic patients. Clin Infect Dis 2021;73: e4616–e4626.
65. Walsh TL, Bremmer DN, Moffa MA, et al. Impact of an antimicrobial stewardship program-bundled initiative utilizing Accelerate Pheno system in the management of patients with aerobic gram-negative bacilli bacteremia. Infection 2021; 49:511–519.
66. Wang YC, Ku WW, Yang YS, et al. Is Polymicrobial Bacteremia an Independent Risk Factor for Mortality in Acinetobacter baumannii Bacteremia?. J Clin Med. 2020;9(1):153.
67. McKenzie, F.E. Case mortality in polymicrobial bloodstream infections. J. Clin. Epidemiol. 2006, 59, 760–761.
68. Pavlaki M, Poulakou G, Drimousis P, et al. Polymicrobial bloodstream infections: Epidemiology and impact on mortality. J Glob Antimicrob Resist. 2013;1(4):207-212.
69. Roberts, F.J.; Geere, I.W.; Coldman, A. A three-year study of positive blood cultures, with emphasis on prognosis. Rev. Infect. Dis. 1991, 13, 34–46.
70. Ilavska I, Pichna P, Stopkova K, et al. Polymicrobial bacteremia in cancer patients: analysis of risk factors, etiology and outcome in 214 episodes. Int J Antimicrob Agents. 1996;7(2):101-107.
71. Aliaga L, Mediavilla JD, Llosá J, Miranda C, Rosa-Fraile M. Clinical significance of polymicrobial versus monomicrobial bacteremia involving Pseudomonas aeruginosa. Eur J Clin Microbiol Infect Dis. 2000;19(11):871-874.
72. Liu Q, Wu J, Wang Z, Wu X, Wang G, Ren J. Polymicrobial Bacteremia Involving Klebsiella pneumoniae in Patients with Complicated Intra-Abdominal Infections: Frequency, Co-Pathogens, Risk Factors, and Clinical Outcomes. Surg Infect (Larchmt). 2019;20(4):317-325.