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BIOCHEMICAL PREDICTORS OF ANTIMICROBIAL RESISTANCE IN HOSPITALIZED PATIENTS WITH GRAM-NEGATIVE BACTERIAL INFECTIONS

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ABSTRACT

Background: Antimicrobial resistance in Gram-negative pathogens is an escalating problem in hospital practice, particularly when multidrug-resistant (MDR) infection is not recognized before empirical antimicrobial therapy is selected. A readily available biochemical profile that identifies patients with a higher probability of MDR infection could strengthen early clinical risk assessment while definitive susceptibility testing is pending.

Objective: To determine whether routinely measured biochemical and inflammatory markers are independently associated with MDR Gram-negative bacterial infection in hospitalized adults and to assess their discriminatory performance for early risk stratification.

Methods: In this prospective cohort study, 150 hospitalized adults with culture-confirmed Gram-negative bacterial infection were evaluated between January 2025 and January 2026. C-reactive protein (CRP), procalcitonin (PCT), serum albumin, lactate, creatinine, total bilirubin, and the CRP-to-albumin ratio (CAR) were assessed in relation to resistance status. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Independent associations were examined with multivariable logistic regression, and discriminatory performance was quantified using receiver operating characteristic (ROC) analysis.

Results: MDR organisms were recovered from 88 of 150 patients (58.7%). *Escherichia coli* was the most frequently isolated pathogen, whereas *Acinetobacter baumannii* showed the greatest organism-specific proportion of MDR. Relative to the non-MDR group, patients with MDR infection had higher median CRP (128 vs. 74 mg/L), PCT (3.4 vs. 1.0 ng/mL), lactate (2.8 vs. 1.9 mmol/L), and CAR (46.1 vs. 22.0), together with lower serum albumin (2.8 vs. 3.4 g/dL). After multivariable adjustment, PCT ≥2.0 ng/mL (aOR 2.71), CAR ≥35 (aOR 2.54), and albumin <3.0 g/dL (aOR 2.19) remained independently associated with MDR infection. The combined biochemical model yielded an AUC of 0.86, which increased to 0.89 when major clinical risk factors were incorporated.

Conclusion: A biochemical pattern characterized by elevated PCT, increased CAR, and hypoalbuminemia was independently associated with MDR Gram-negative infection. These routinely available measures may add clinically useful information to exposure history and microbiological assessment, but they should be interpreted as components of an integrated risk model rather than as substitutes for definitive susceptibility testing.

KEYWORDS: antimicrobial resistance; multidrug-resistant organisms; Gram-negative infection; procalcitonin; CRP-to-albumin ratio; risk stratification

INTRODUCTION

Antimicrobial resistance (AMR) is changing the clinical approach to managing severe bacterial infection and has a particularly high impact on in-hospital patients with Gram-negative bacteria [1]. The higher the resistance, the less likely standard empirical therapy is to provide the patient with active early therapy, exposing patients to treatment delay, longer hospital stays, higher resource consumption, and possibly poorer clinical outcomes. Gram-negative bacteria are especially flexible: resistance can be acquired and disseminated in a number of ways, such as the expression of extended-spectrum β-lactamases and carbapenemases, active efflux, decreased membrane permeability, and alteration of antimicrobial target molecules [2]. This translates to uncertainty about which therapy is most effective, which is most prominent in the period between clinical recognition of infection and definitive antimicrobial susceptibility information.

E. coli, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are among the organisms most commonly associated with hard-to-treat hospital infections [3]. Antibiotic exposure history, repeated healthcare contacts, invasive devices, intensive care contacts, comorbidity burden, and acquisition in healthcare facilities are risk factors for encountering a resistant isolate. Although clinically informative, these risk factors do not completely solve the decision problem at presentation, as the culture and susceptibility testing requires time and may not be available at the time of choosing the initial antimicrobial regimen [4,5]. Therefore, a method for refining pre-susceptibility risk without bypassing microbiological confirmation would have practical value.

The most frequently measured biochemical variables may reflect other aspects of the host response to infection. C-reactive protein (CRP) is an indicator of the activity of the acute-phase inflammatory process, while procalcitonin (PCT) is associated with a more systemic bacterial inflammatory process [6]. Lactate can be used to assess physiological stress and inadequate tissue perfusion, and creatinine can be used to detect if renal dysfunction is present. Serum albumin is a protein that is affected by nutritional reserve, systemic inflammation, redistribution, capillary leakage, and severity of illness. The use of an inflammatory marker and albumin in the CRP-to-albumin ratio (CAR) would therefore provide a better integration of inflammatory intensity with host reserve than either would alone [7]. These variables are readily available, easy to get, and already part of the basic inpatient evaluation.

The majority of biomarker studies of bacterial infection target diagnosis, severity estimation, treatment guidance, or prognosis, but not the likelihood of antimicrobial resistance per se [8]. However, delayed effective MDR therapy, increased bacterial load, more complex healthcare exposures, and ongoing systemic inflammation can coexist with MDR infection. These pathways provide a biologically plausible basis for differences in routine biochemical profiles between patients with MDR and non-MDR infections, while also emphasizing that any observed association may reflect both resistance and the clinical context in which resistant infection occurs [9]. Evaluating biomarkers in a multivariable framework is therefore important to distinguish independent signal from simple correlation with illness severity or healthcare exposure [9].

This question is particularly relevant in settings where rapid molecular resistance assays are not consistently available. In such environments, a parsimonious panel assembled from routinely obtained inflammatory, metabolic, and organ-function measurements could be used to refine early risk estimation while culture-based susceptibility results are pending. A multimarker strategy is also conceptually preferable to reliance on a single analyte because different markers reflect different components of host physiology and may provide incremental discriminatory information when combined [10,11].

Accordingly, this study evaluated the association between routinely available biochemical markers and MDR status in hospitalized adults with culture-confirmed Gram-negative bacterial infection. The prespecified panel comprised CRP, PCT, serum albumin, lactate, creatinine, total bilirubin, and CAR. The principal objectives were to identify variables independently associated with MDR infection after multivariable adjustment and to quantify the discrimination of individual markers and combined biochemical-clinical models for early risk stratification.

MATERIALS AND METHODS

Study Design and Setting:

This prospective cohort study was undertaken at the Department of Biochemistry, Bakhtawar Amin Medical and Dental College, Multan, Pakistan, and the Institute of Molecular Biology and Biotechnology (IMBB)/Centre for Research in Molecular Medicine (CriMM), The University of Lahore, Lahore, Pakistan, between January 2025 and January 2026. The analytic framework focused on hospitalized adults with microbiologically confirmed Gram-negative infection and examined whether biochemical measurements obtained around the index infectious episode were associated with multidrug-resistant (MDR) status.

Study Population:

Eligible participants were adults ≥18 years who were hospitalized with a clinically suspected infection and subsequently had a clinically significant Gram-negative organism isolated from an appropriate specimen. Only the first infectious episode in each person was analysed to avoid the clustering of repeated events within individuals. Polymicrobial infection (with Gram-positive bacteria or fungi, a culture diagnosis of colonization or contamination was not considered an infection, incomplete antimicrobial susceptibility data, or lack of baseline biochemical measurements needed for the study analyses were excluded. Finally, 150 participants with an evaluable index infection were included.

Sampling and Clinical Data Collection:

Patients were selected in a consecutive fashion with confirmation of clinically relevant Gram-negative growth. A structured collection form was used to record demographic, exposure, and clinical data on standardised ascertainment for all the participants. Recorded variables comprised age, sex, major co-morbidities, anatomical source of infection, intensive care unit (ICU) admission, prior hospitalization, use of invasive devices, healthcare-associated acquisition, and prior exposure to antibacterials. Operationally, prior antibiotic use was defined as the use of at least 48 hours of

systemic antibacterial therapy within the 3 months before the study. These variables were chosen because they might affect the likelihood of resistant infection, as well as the biochemical reaction to acute illness.

Specimen Collection and Microbiological Assessment:

Clinical specimens were collected based on the focus of the suspected infection, which included blood, urine, respiratory material, other samples as clinically indicated, and wound/tissue samples. Isolation and identification of clinically significant Gram-negative bacteria were performed by standard microbiological methods. The organisms of primary interest were *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and other clinically significant Enterobacterales. Isolates considered to represent colonization or contamination were not added to the analytic cohort, and microbiological results were analyzed with clinical presentation.

Routine laboratory standard antimicrobial susceptibility testing was done. Susceptibility was reported for clinically relevant antimicrobial categories, including β-lactams, cephalosporins, fluoroquinolones, aminoglycosides, carbapenems, and other drugs tested as appropriate to the organisms recovered. When indicated, ESBL production and carbapenem resistance were documented. The susceptibility profile of the index isolate was applied to categorize each infection for the primary study outcome.

Definition of Multidrug Resistance:

The primary microbiological outcome was infection with an MDR GN organism. This operational definition, which was applied during the study, was used to define MDR as being non-susceptible to at least one antimicrobial agent in three or more relevant antimicrobial categories. All those isolates with a value below this threshold were considered non-MDR and were used as a reference group for comparative and regression analyses. This binary classification was carried out on the first isolate of the participant that met the criteria.

Biochemical Assessment:

Blood samples taken at the first assessment of the index infectious episode were used for biochemical measurements. If more than one result was available, the result that temporally most closely matched the microbiological specimen collection date and occurred prior to significant changes in antimicrobial therapy was selected for use, to maximize temporal proximity and facilitate temporal alignment between the measurement of biomarker and assessment of resistance. Main analytes were CRP, PCT, serum Alb, lactate, serum Creat, and total Bilirubin. These markers, in combination, indicated inflammatory activity, metabolic stress, organ dysfunction, and host physiological reserve.

The formula used for calculating CAR was: CRP (mg/L)/Albumin (g/dL). Prespecified thresholds for categorical predictive analyses were: PCT ≥2.0 ng/mL, CRP ≥100 mg/L, lactate ≥2.5 mmol/L, albumin<3.0 g/dL, creatinine ≥1.5 mg/dL, and CAR ≥35. Conventional automated biochemical assays were used in laboratory testing according to the internal quality-control system of the individual laboratories. To retain the consistency of the interpretation, the same units and threshold definitions were used when the data were used for descriptive, regression, and ROC analyses.

Outcome Measures:

The primary outcome was the MDR status of the index gram-negative infection. Secondary clinical outcomes included the need for intensive care, length of stay, adjustment or change of antimicrobial therapy following susceptibility results, and death in hospital. Biochemical parameters were compared across the two groups of MDR and non-MDR, and the main inferential analysis tested the independence of the biochemical parameters from the relevant clinical parameters in the context of the MDR infection.

Statistical Analysis:

Continuous variables were examined for distributional characteristics and summarized as mean ± standard deviation (SD) when approximately normally distributed or as median with interquartile range (IQR) when non-normal. Categorical variables were reported as counts and percentages. Between-group comparisons used the independent-samples t-test for normally distributed continuous data and the Mann-Whitney U test for non-normally distributed data. Categorical variables were compared with the chi-square test or Fisher's exact test when expected cell counts made the latter more appropriate. All statistical tests were two-sided.

Variables with clinical relevance or a univariable association at $p<0.10$ were considered for entry into multivariable binary logistic regression. Adjusted odds ratios (aORs) with 95% confidence intervals (Cis) were used to quantify independent associations with MDR infection, and candidate variables were assessed for multicollinearity before final model construction. ROC analysis was then used to characterize discrimination for individual biomarkers and combined models. Area under the ROC curve (AUC) with 95% CI was reported, together with sensitivity and specificity for the combined biochemical model. Statistical significance was defined as a two-sided $p<0.05$; interpretation emphasized effect estimates and confidence intervals in addition to p values.

Ethical Considerations:

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Approval was obtained from the relevant institutional ethics committee before study initiation. Written informed consent was secured from participants or, where appropriate, from legally authorized representatives. Identifying information was removed before analysis, study data were handled confidentially, and results were analyzed and reported in aggregate form to protect participant privacy.

RESULTS

Participant Characteristics and Clinical Profile:

The final analysis included 150 hospitalized adults with culture-confirmed Gram-negative bacterial infection. Mean age was 56.7 ± 15.2 years, and the cohort comprised 85 men (56.7%) and 65 women (43.3%). MDR organisms were identified in 88 participants (58.7%), whereas 62 (41.3%) had isolates that did not meet the MDR definition. Thus, more than half of the evaluable index infections in this cohort were caused by organisms with multidrug resistance.

Patients in the MDR group were older than those in the non-MDR group (58.6 ± 14.8 vs. 54.1 ± 15.4 years; p=0.041), while the distribution of sex was similar (p=0.701). Several established healthcare-exposure variables clustered in the MDR group: previous antibiotic exposure was present in 68.2% versus 40.3% (p=0.001), invasive-device use in 61.4% versus 35.5% (p=0.002), healthcare-associated infection in 64.8% versus 38.7% (p=0.002), and ICU admission in 47.7% versus 25.8% (p=0.007). Median hospital stay was also longer with MDR infection (12 vs. 8 days; p<0.001). In-hospital mortality was higher numerically in the MDR group (17.0% vs. 6.5%), but the between-group difference did not reach the prespecified threshold for statistical significance (p=0.056). Detailed baseline characteristics are presented in Table 1.

Table 1. Demographic and Clinical Characteristics Stratified by MDR Status

Variable	MDR group (n=88)	Non-MDR group (n=62)	p-value
Age, years, mean ± SD	58.6 ± 14.8	54.1 ± 15.4	0.041
Male, n (%)	51 (58.0)	34 (54.8)	0.701
Female, n (%)	37 (42.0)	28 (45.2)	—
Diabetes mellitus, n (%)	36 (40.9)	18 (29.0)	0.136
Hypertension, n (%)	39 (44.3)	22 (35.5)	0.281
Previous antibiotic exposure, n (%)	60 (68.2)	25 (40.3)	0.001
Invasive-device use, n (%)	54 (61.4)	22 (35.5)	0.002
Healthcare-associated infection, n (%)	57 (64.8)	24 (38.7)	0.002
ICU admission, n (%)	42 (47.7)	16 (25.8)	0.007
Hospital stay, days, median (IQR)	12 (8–18)	8 (6–13)	<0.001
In-hospital mortality, n (%)	15 (17.0)	4 (6.5)	0.056

Values are mean ± SD, median (IQR), or n (%). Age was compared with the independent-samples t-test, hospital stay with the Mann-Whitney U test, and categorical variables with the chi-square test or Fisher's exact test as appropriate. ICU: intensive care unit; MDR: multidrug-resistant.

Microbiological Characteristics:

Escherichia coli was the most prevalent organism (34.7%) with 52 out of 150 isolates. *Klebsiella pneumoniae* was recovered from 43 patients (28.7%), *Acinetobacter baumannii* from 26 (17.3%), *Pseudomonas aeruginosa* from 21 (14.0%), and other Enterobacterales from 8 (5.3%). This distribution shows that the cohort consisted of both Enterobacterales and non-fermenting Gram-negative pathogens with different resistance profiles in terms of clinical aspects.

However, the percentage that met the MDR definition was quite different for each organism. The highest frequency of MDR was found in *Acinetobacter baumannii* (84.6%) (22/26), followed by *Klebsiella pneumoniae* (67.4%) (29/43). Twenty-four of 52 *Escherichia coli* (46.2%) and 10 of 21 *Pseudomonas aeruginosa* (47.6%) isolates were MDR. The distribution of MDR and non-MDR isolates in the complete organism is summarized in Table 2.

Urinary tract infection was the most common source of infection (34.0%, 51 cases). Thirty-seven cases (24.7%) were bloodstream infections, 34 (22.7%) were respiratory tract infections, 20 (13.3%) were wound or soft-tissue infections, and 8 (5.3%) were from other anatomical sources. These source categories were documented for the index episode and used to give clinical context for interpretation of the resistance and biomarker analyses.

Table 2. Distribution of Gram-Negative Pathogens by MDR Classification

Organism	Total isolates, n (%)	MDR isolates, n (%)	Non-MDR isolates, n (%)
Escherichia coli	52 (34.7)	24 (46.2)	28 (53.8)
Klebsiella pneumoniae	43 (28.7)	29 (67.4)	14 (32.6)
Acinetobacter baumannii	26 (17.3)	22 (84.6)	4 (15.4)
Pseudomonas aeruginosa	21 (14.0)	10 (47.6)	11 (52.4)
Other Enterobacterales	8 (5.3)	3 (37.5)	5 (62.5)
Total	**150 (100)**	**88 (58.7)**	**62 (41.3)**

Percentages in the MDR and non-MDR columns are calculated within each organism. MDR: multidrug resistant.

Biochemical Profile According to Resistance Status:

Biochemical profiles differed materially between resistance groups. Median CRP was 128 mg/L (IQR 92-184) in patients with MDR infection compared with 74 mg/L (IQR 43-116) in the non-MDR group ($p<0.001$). PCT showed a similarly pronounced separation, with median concentrations of 3.4 ng/mL (IQR 1.5-8.1) and 1.0 ng/mL (IQR 0.4-2.4), respectively ($p<0.001$). Lactate was also higher in the MDR group, with a median of 2.8 mmol/L (IQR 2.0-4.1) versus 1.9 mmol/L (IQR 1.4-2.8; $p=0.002$), indicating greater physiological stress among patients with resistant infection.

Serum albumin showed the opposite pattern and was lower in the MDR group (2.8 ± 0.6 vs. 3.4 ± 0.5 g/dL; $p<0.001$). Median creatinine was higher with MDR infection (1.46 vs. 1.05 mg/dL; $p=0.011$), whereas total bilirubin did not differ significantly between groups. CAR produced one of the widest separations among the evaluated markers: the median ratio was 46.1 (IQR 30.4-67.9) in the MDR group and 22.0 (IQR 13.1-35.8) in the non-MDR group ($p<0.001$). Group distributions and adjusted associations are reported together in Table 3.

In the multivariable logistic model, PCT ≥2.0 ng/mL remained independently associated with MDR infection (aOR 2.71; 95% CI 1.30-5.64; $p=0.008$). CAR ≥35 was also independently associated with MDR status (aOR 2.54; 95% CI 1.20-5.37; $p=0.015$), as was albumin <3.0 g/dL (aOR 2.19; 95% CI 1.03-4.67; $p=0.042$). By contrast, CRP ≥100 mg/L, lactate ≥2.5 mmol/L, and creatinine ≥1.5 mg/dL were positively associated at the point-estimate level but did not retain independent statistical significance after adjustment. These findings suggest that the strongest biochemical signal was concentrated in PCT, CAR, and hypoalbuminemia rather than in every marker that differed on unadjusted comparison (Table 3).

Table 3. Biochemical Profiles and Adjusted Associations With MDR Infection

Parameter	MDR group (n=88)	Non-MDR group (n=62)	Adjusted OR (95% CI)	p-value
CRP, mg/L	128 (92–184)	74 (43–116)	1.71 (0.80–3.65)	0.164
Procalcitonin, ng/mL	3.4 (1.5–8.1)	1.0 (0.4–2.4)	2.71 (1.30–5.64)	0.008
Lactate, mmol/L	2.8 (2.0–4.1)	1.9 (1.4–2.8)	1.89 (0.91–3.94)	0.088
Albumin, g/dL	2.8 ± 0.6	3.4 ± 0.5	2.19 (1.03–4.67)	0.042
Creatinine, mg/dL	1.46 (0.98–2.21)	1.05 (0.79–1.55)	1.82 (0.86–3.85)	0.117
Total bilirubin, mg/dL	0.9 (0.6–1.5)	0.8 (0.5–1.3)	—	0.218
CRP-to-albumin ratio	46.1 (30.4–67.9)	22.0 (13.1–35.8)	2.54 (1.20–5.37)	0.015

Values are mean ± SD or median (IQR). Group comparisons used the independent-samples t-test or Mann-Whitney U test as appropriate. Adjusted ORs correspond to the prespecified thresholds: CRP ≥100 mg/L, PCT ≥2.0 ng/mL, lactate ≥2.5 mmol/L, albumin <3.0 g/dL, creatinine ≥1.5 mg/dL, and CAR ≥35. OR: odds ratio; CI: confidence interval; CRP: C-reactive protein; CAR: CRP-to-albumin ratio.

Predictive Performance of Biochemical Markers:

CAR discriminated best among individual biochemical markers on ROC analysis, with an AUC of 0.79 (95% CI 0.72-0.86). PCT yielded an AUC of 0.76 (95% CI 0.68-0.83), while CRP yielded an AUC of 0.74 (95% CI 0.66-0.82). Moderate stand-alone discrimination was thus obtained for each marker, and CAR numerically outperformed the other single biochemical measures evaluated.

The addition of PCT, CAR, albumin and lactate resulted in an AUC of 0.86 (95% CI 0.80-0.92). In this biochemical model, the sensitivity and specificity for MDR infection were 81.8% and 77.4%, respectively, at the reported operating point. The AUC then rose to 0.89 (95% CI 0.84-0.94) when biochemical data were combined with well-defined clinical exposure variables, suggesting greater discriminatory ability when biochemical data were combined with established clinical exposure variables. The ROC curves of the individual and combined model are shown in Figure 1.

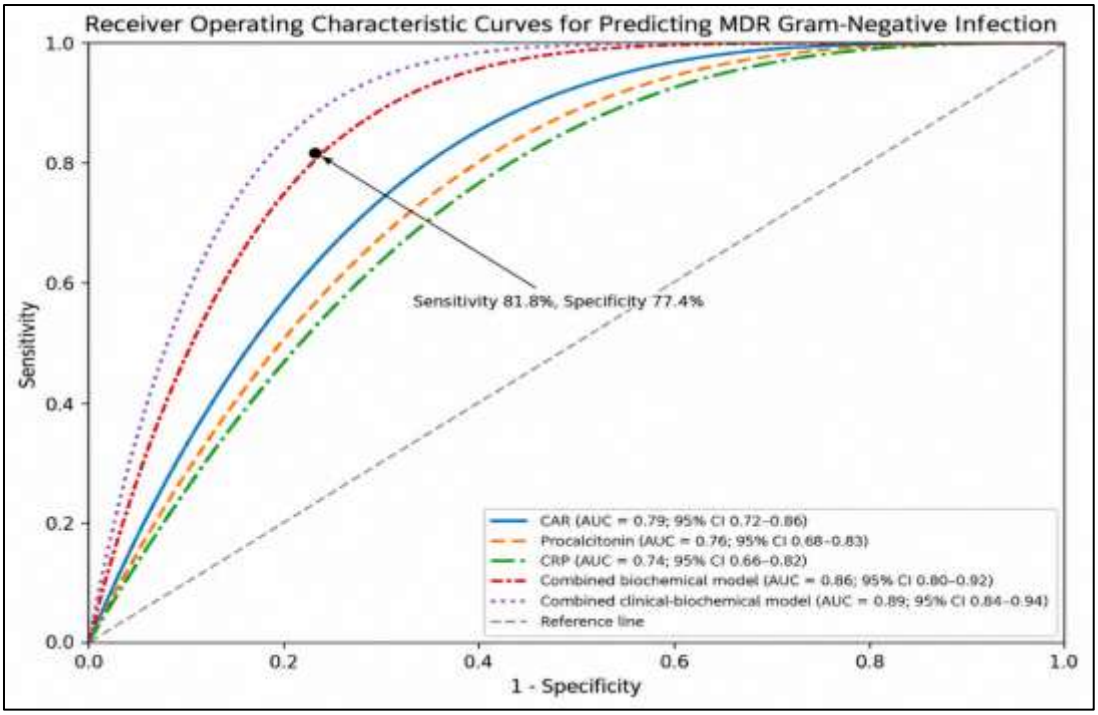

Figure 1. Receiver Operating Characteristic Curves for Discrimination of MDR Gram-Negative Infection

Overall, the predictive analyses revealed a unique inflammatory and metabolic profile associated with MDR infection. PCT, CAR, and low albumin remained independently associated in the adjusted model, and the best AUC was achieved with the addition of biochemical data and past antibiotic use and HAC. These results help to support integrated risk estimation rather than interpretation of an individual lab result alone.

DISCUSSION

This prospective cohort identified a high burden of MDR Gram-negative infection and, more importantly, showed that a subset of routinely available biochemical markers contained information that was not fully captured by conventional clinical characteristics [1,2]. MDR organisms represented 58.7% of isolates, with the greatest organism-specific proportions in *Acinetobacter baumannii* and *Klebsiella pneumoniae*. The MDR group also experienced more prior antibiotic exposure, use of invasive devices, HAA, admission to ICU, and LOS. This is similar to the known association of antimicrobial pressure, health care contact and invasive care, and selection or acquisition of resistant Gram-negative pathogens [3]. Thus, the findings with the biomarkers should not be seen as evidence that inflammation directly leads to antimicrobial resistance, but rather in the context of the above epidemiology.

One of the strongest independent biochemical correlates of MDR status was PCT (AOR = 2.7 for a concentration ≥2.0 ng/mL). Although the ability of PCT to differentiate resistant from susceptible infection remains far from certain, PCT has been proven as a marker of systemic bacterial inflammation [4]. This elevated concentration may be a combination of higher bacterial load, delayed onset of appropriate antimicrobial therapy, more intense systemic host response, and/or the complexity of the patient that accompanies a healthcare-associated infection. Importantly, PCT does not determine a resistance mechanism, and should not be regarded as a microbiological surrogate. Its usefulness is more likely to be as a part of a pre-susceptibility risk profile, along with history of exposure and clinical severity [5,6].

CAR was the most discriminative of all the individual biomarkers and was also independently associated with MDR infection. Biologically, it is interesting because it combines two acute-phase processes: CRP rising as a function of inflammatory signaling and albumin often falling due to the effects of altered hepatic synthesis, redistribution, capillary leakage, nutritional status, and systemic illness [7]. When both are taking place simultaneously, a clinically relevant contrast can thus be magnified by a composite ratio. However, in this cohort, the magnitude of CRP was significantly higher with MDR infection on simple comparison, but lost its independent meaning after adjustment, while CAR was still informative [8,9]. This difference indicates that the ratio might reflect a more general host-state signal than CRP alone and would need to be confirmed in an externally validated cohort before its use in clinical practice as a fixed CAR.

The third biochemical measurement that was independently associated with MDR infection was hypoalbuminemia. Low levels of albumin are not likely to be resistance-specific, but may represent a chronic disease, inadequate nutritional reserve, systemic inflammation, capillary leak, or chronic healthcare exposure. Many of the same factors

predispose to resistant infection and exacerbate the physiologic effects of acute sepsis. Thus, the association observed in this study may be more a reflection of host frailty or cumulative illness burden per se rather than resistance biology. The association observed in this study may therefore be more a reflection of host frailty or cumulative illness burden per se rather than resistance biology. [10,11] This interpretation has clinical relevance as it suggests that albumin should not be used as a stand-alone indicator for antimicrobial use; however, it can be included in risk assessment models.

Multivariable adjustment revealed that lactate and creatinine were elevated in the MDR infection group, but they were not independent risk factors. However, both markers are highly affected by acute illness severity and organ dysfunction, and illness intensity could account for the raw association. However, total bilirubin values were not significantly different between the groups and did not seem to be obviously discriminatory in this population. Thus, the overall pattern is in favour of a multidimensional model where inflammatory burden, host reserve, metabolic stress, and health care exposure are all taken into account, and not assuming that every abnormal biochemical value reflects resistance directly [12,13].

The organism distribution provides extra clinical context. The highest proportion of MDR was found in *Acinetobacter baumannii* and *Klebsiella pneumoniae*, which, along with other pathogens, are of particular importance in the development of HAIs and are able to accumulate multiple resistance determinants. A combination of carbapenemases, ESBLs, altered OMPs, and efflux mechanisms can co-exist and significantly reduce therapeutic choices [14,15]. The prevalence of MDR in these organisms underscores the need for local susceptibility surveillance, infection prevention practice, antimicrobial stewardship, and rapid identification of organisms. These measures can only be supplemented by a biochemical risk model and should not be used instead of testing for susceptibility to the isolate.

ROC analyses are clinically informative since they demonstrate incremental discrimination over increasingly integrated models. The biochemical model alone yielded a moderate AUC, while the same with any one of the three factors (CAR, PCT, or CRP) yielded a similar moderate AUC, but the biochemical model with all three combined attained an AUC of 0.86. Incorporation of prior antibiotic use and acquisition of HA-MRSA raised the AUC to 0.89. This pattern fits the concept that resistance risk is multi-faceted and that laboratory markers will be most effective when used in conjunction with known epidemiological risk factors [16,17]. It also warns against giving too much significance to a single best cut-off: For a model designed to be used in clinical practice, calibration, discrimination, and decision thresholds would need to be validated in another population prior to implementation.

The results could impact empirical antimicrobial decision-making, especially prior to the arrival of final susceptibility results. An antibiotic-naive hospitalized patient with markedly elevated PCT, a high CAR, hypoalbuminemia, and recent antibiotic therapy should have a higher suspicion for MDR Gram-negative infection [11,18]. Clinically, that signal might be used to consider earlier infectious-disease review, consideration of more rapid phenotypic or molecular susceptibility testing when available, and more frequent reassessment of empirical therapy when microbiological information becomes available. However, it should not be a blanket prescription for over-reaching broad-spectrum escalation, as over-expansion of antimicrobial use can lead to greater toxicity, ecological pressures, and resistance selection [19]. It is this targeted risk enrichment, with stewardship care in mind, that is the most defensible 'role'.

There are several limitations to these results. First, the moderate sample size limited organism-specific analyses and limited the precision of estimation of subgroup effects. Second, resistance was phenotypically characterized, and no attempt was made to systematically examine molecular determinants; thus, the relationship of biomarker patterns to specific resistance genes and mechanisms cannot be established. Third, factors besides resistance, such as comorbid disease, renal function, nutritional status, severity of the infection, and the time of sample collection, affect the results for PCT, CRP, albumin, lactate, and creatinine. Multivariable adjustment thus cannot rule out the possibility of residual confounding. Lastly, the prespecified biomarker thresholds and combined model were developed and validated within the same cohort and need to be validated in larger external cohorts - ideally multicenter and with molecular resistance characterization - before routine clinical use [20].

CONCLUSION

MDR organisms were common in this population of hospitalized adults with Gram-negative culture-positive infection, and were associated with a unique biochemical profile. Multivariable adjustment confirmed that PCT ≥2.0 ng/mL, CAR ≥35, and albumin <3.0 g/dL were independently associated with MDR status, and that recent antibiotic use and healthcare-associated acquisition provided clinically important discriminatory information. The biochemical variables together yielded good discrimination, and the inclusion of the clinical exposure variables also improved the discrimination. These results are indicative of the value of routine biochemistry as an adjunct to rather than an alternative to microbiological diagnosis and susceptibility testing. The most feasible clinical use is for incorporation into a comprehensive early risk scoring system to help triage the level of diagnostic investigation and antimicrobial review during the period of time awaiting definitive results. The proposed thresholds and/or model need to be validated in larger, multicenter populations, and molecular mechanisms of resistance need to be characterized before it can be translated into a generalizable decision tool.

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

Funding: No specific funding was received.

Author Contributions: A.M.: conception, supervision, manuscript review; S.Z.: laboratory work and interpretation; A.K.: microbiology and data collection; S.H.: biochemical assessment and drafting; A.S.: methodology and statistical analysis; A.Z.: antimicrobial susceptibility assessment; A.Y.M.: analysis and literature review; T.N.: clinical data collection and manuscript review. All authors approved the final manuscript.

Acknowledgments: The authors acknowledge the participating clinical and laboratory staff.

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