scientific reports

Check for updates

OPEN

Evaluating a pathogen-specific IgG binding assay for rapid detection of healthcare-associated infections

Asiye Karakullukçu[1]✉, Mustafa Akker[2], Mert Ahmet Kuşkucu[3], Gökhan Aygün[4] & Yalım Dikmen[5]

Rapid and accurate diagnosis of healthcare-associated infections (HAIs) is an unmet need for improving outcomes in intensive care units (ICUs). Traditional culture-based methods, while the gold standard, are time-consuming and can delay therapeutic interventions. In this study, we evaluated the diagnostic utility of pathogen-specific immunoglobulin G (IgG) binding levels using an enzyme-linked immunosorbent assay (ELISA). We measured IgG binding against pathogens including *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Staphylococcus aureus*. Diagnostic performance was evaluated through receiver operating characteristic (ROC) curve analysis, with culture results as the reference. The assay demonstrated an overall diagnostic accuracy of 83.2%, with a sensitivity of 85.4%, a specificity of 81.4%, and an area under the curve (AUC) of 0.910. Pathogen-specific cutoff values ranged from 0.918 to 1.534. Especially, *A. baumannii* showed the highest performance metrics, achieving a sensitivity of 94.7%, a specificity of 93.6%, and an AUC of 0.975. The pathogen-specific IgG binding levels can offer a novel and effective diagnostic tool for the initial assessment of HAIs, enhancing early detection and improving patient management across healthcare settings.

Keywords Healthcare-associated infections, Pathogen-specific IgG, Intensive care unit, Rapid diagnostic test, Enzyme-linked immunosorbent assay, Antibiotic stewardship

Healthcare-associated infections (HAIs) are a significant global challenge, with an average mortality rate of 10% among affected patients, especially in intensive care units (ICUs). In high-income countries, HAIs impact up to 30% of ICU patients and are frequently associated with comorbidities, immunosuppression, and invasive devices. Common causative agents include *Escherichia coli*, *Pseudomonas aeruginosa*, *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus*, and vancomycin-resistant *Enterococci*[1].

The conventional gold standard for diagnosing HAIs is the culture method, which requires 48 to 72 h to provide results. This delay can lead to overuse of broad-spectrum antibiotics, extended hospital stays, increased morbidity, and increased healthcare costs. Consequently, there is a critical need for more rapid diagnostic methods to facilitate timely and precise therapeutic interventions to improve patient management and outcomes[2]. Rapid techniques such as matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), EUCAST Rapid Antimicrobial Susceptibility Testing (RAST), and multiplex polymerase chain reaction (PCR) panels accelerate identification, but they detect microbial components rather than the host response and may not distinguish colonization from real infection or diagnose active infection under antibiotic suppression[3–6].

Recognizing these ongoing challenges, recent studies have focused on novel biomarkers for early detection of bacterial infections and have developed rapid, point-of-care tests for multidrug-resistant organisms[7,8]. While these technologies offer valuable insights, they still face limitations in sensitivity, specificity, and broader clinical applicability. Furthermore, the increasing prevalence of antibiotic resistance calls for enhanced diagnostic techniques that can not only quickly identify pathogens but also inform appropriate antibiotic therapy[9].

Immunoglobulin G (IgG) plays a pivotal role in host defense by binding specifically to bacterial antigens and promoting pathogen clearance. This key feature underlines the diagnostic potential of IgG-based assays for

[1]Faculty of Medicine, Department of Medical Microbiology, Istanbul Health and Technology University, Istanbul, Turkey. [2]Department of Intensive Care, Istinye University Medical Park Gaziosmanpasa Hospital, Istanbul, Turkey. [3]Faculty of Medicine, Department of Medical Microbiology, Koç University, Istanbul, Turkey. [4]Cerrahpasa Faculty of Medicine, Department of Medical Microbiology, Istanbul University-Cerrahpasa, Istanbul, Turkey. [5]Cerrahpasa Faculty of Medicine, Department of Anesthesiology and Reanimation, Istanbul University-Cerrahpasa, Istanbul, Turkey. ✉email: asiyekarakullukcu@gmail.com

early bacterial infection detection[10,11]. Enzyme-linked immunosorbent assay (ELISA) techniques leverage this mechanism, providing a means to distinguish infected from non-infected patients. ELISA can be performed using standard laboratory equipment and delivers results within a few hours, thereby supporting early clinical decision-making[12].

In this study, we introduced a diagnostic approach utilizing ELISA to measure IgG binding levels against common pathogens such as *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Enterococcus faecium*. By comparing the efficacy of this method with traditional culture results, we aimed to establish a faster and more precise diagnostic tool, enhancing clinical outcomes through earlier and more targeted interventions in ICU settings.

Methods

Study settings

The study was conducted in the ICUs of Cerrahpaşa University Hospital and Istanbul Şişli Hamidiye Etfal Training and Research Hospital, two tertiary care hospitals in Istanbul. We included ICU patients who were admitted without infections and were not immunosuppressed or pregnant. Patients who developed infections with target pathogens during their stay were included. The target pathogens were *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Enterococcus faecium*. We selected these pathogens based on local epidemiology and clinical impact in the included ICUs. Non-target organisms were defined as microbial species not covered by the seven-pathogen IgG panel.

Sample collection

Serum samples for the IgG binding assay were collected within 24 h of obtaining culture samples from different body sites, including blood, endotracheal aspirates (ETA), bronchoalveolar lavage (BAL), urine, sterile body fluids, and tissues. Sample collection procedures were blinded to culture results to mitigate bias. Samples were preserved at -80 °C until processed. ELISA assays were performed weekly to ensure consistency [12].

We recorded demographic and clinical data for each patient, including age, sex, dates of admission and sample collection, and the duration of the hospital stay, using paper-based case report forms.

Bacterial strains and sample processing

Routine culture samples were processed using standard microbiological methods in the Clinical Microbiology Laboratory. Pathogens were identified via MALDI-TOF MS (Bruker Corporation). We defined specific criteria for significant bacterial growth, including the requirement for simultaneous growth in two or more sets of blood cultures from different veins; endotracheal aspirate (ETA) cultures with $\geq 10^5$ colony-forming units (CFU)/mL; bronchoalveolar lavage (BAL) cultures with $\geq 10^4$ CFU/mL; urine samples with $\geq 10^5$ CFU/mL; and pathogen growth of $\geq 10^3$ CFU/mL in sterile body fluids or tissues without normal flora[13]. Samples with over four pathogen types were excluded to prevent confounding.

IgG binding assay

The IgG binding assays were conducted independently of culture results to prevent bias. We used ATCC pathogen strains adjusted to a 0.5 McFarland standard for assay preparations. IgG binding levels were quantified using ELISA in optical density (OD) units. A 100mL bacterial suspension at 10 µg/mL was prepared from 1.25 mL of these cultures in 98.75 mL carbonate-bicarbonate buffer and added to 96-well plates (100 µL/well). The plates were incubated at 37 °C for one hour, washed with phosphate - buffered saline (PBS) containing 0.1% Tween 20, then blocked with 1% bovine serum albumin (BSA) in PBS (200 µL/well), and incubated for another hour at 37 °C. After subsequent washing, a 1:2 dilution of patient samples in 1% BSA (100 µL/well) was added and incubated for an additional hour at 37 °C. Following a final wash, the substrate pNPP (100 µL/well, Sigma P7998) was added and incubated for 20 min at room temperature. The reaction was stopped with 1M H_2SO_4 (100 µL/well), and the absorbance was measured at 405 nm using a Thermo Scientific™ Multiskan GO spectrophotometer, with results calibrated using Thermo Scientific™ SkanIt™ Software version 3.2 (https://www.thermofisher.com/order/catalog/product/5187139). We tested this dilution against dilutions of patient samples (1:100, 1:50, 1:10, and 1:2), finding that 1:2 provided the most consistent results. Serum from patients with confirmed pathogen growth in blood cultures was used as a positive control, and bovine serum albumin was used as a negative control to establish the baseline and assess nonspecific reactions. Assays were performed in triplicate to ensure reproducibility[14]. IgG binding levels were quantified as the mean absorbance at 405 nm (OD_{405}) from triplicate wells. This OD_{405} value was directly proportional to the amount of patient IgG bound to the pathogen antigens in the well and thus served as the quantitative measure of pathogen-specific IgG-binding titer.

Data analysis

Data analysis was conducted utilizing GraphPad Prism™ version 10.5.0 (673) (GraphPad Software, San Diego, CA, USA; https://www.graphpad.com/scientific-software/prism/)[15]. Basic descriptive statistics were employed to outline patient demographics and clinical outcomes. We employed a chi-square test to assess the variance in pathogen distribution and the Kruskal-Wallis test with Dunn's post-hoc analysis to determine differences in IgG binding levels among pathogens. The Brown-Forsythe and Welch ANOVA tests were utilized to compare IgG binding levels across different infection sites. Changes in IgG binding levels over time were assessed using the Wilcoxon matched-pairs signed-rank test and Pearson's correlation coefficient. We employed Welch's test to compare IgG binding efficacy between single and multiple pathogen infections and utilized a two-way ANOVA to analyze the effects of multiple simultaneous pathogens. We evaluated the diagnostic performance of pathogen-specific IgG levels using receiver operating characteristic (ROC) curve analysis, comparing IgG results

to culture outcomes as the reference standard. ROC curves plot sensitivity against 1−specificity for various optical-density (OD) thresholds. The area under the ROC curve (AUC), calculated using Prism software, reflects diagnostic accuracy (values closer to 1.0 indicate better discrimination). Optimal cutoff values were determined using Youden's index (maximum sensitivity + specificity −1). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated at these cutoffs. All results were presented with 95% confidence intervals, both overall and by pathogen type.

Finally, categorical analysis of test reliability involved calculating the Kappa statistic and odds ratio using Fisher's exact test to correlate IgG binding assay results with culture outcomes. All statistical tests were performed, considering a significance threshold set at $p < 0.05$[16].

Results

Demographic and clinical characteristics of ICU patients

A total of 432 serum samples were collected from 225 ICU patients, of which 315 met the study criteria. The average age of the patients was 60.6 years, with an average hospital stay of 14.8 days. The gender distribution included 55% males and 45% females, with an overall mortality rate of 31%. Patients were predominantly from Cerrahpaşa University Hospital (68%), with the remaining 32% from Şişli Etfal Training and Research Hospital.

Pathogen distributions in culture-positive samples

Acinetobacter baumannii was identified as the most prevalent pathogen, detected in 27.7% of culture-positive samples, followed by *E. coli* (17.5%), *P. aeruginosa* (16%), *K. pneumoniae* (11.6%), *E. faecalis* (11.6%), *E. faecium* (10.2%), and *S. aureus* (5.1%). Among non-target organisms, *Corynebacterium spp.* and *Proteus mirabilis* were most common in culture-positive patients.

IgG binding levels across different pathogens and infection sites

Significant differences in IgG binding levels among the target pathogens were observed ($p < 0.0001$, Kruskal-Wallis statistic = 52.61). Dunn's post-hoc tests indicated that *P. aeruginosa* exhibited the highest overall OD values, while both *E. coli* and *E. faecalis* demonstrated significantly higher values than *S. aureus* (Fig. 1).

We stratified each pathogen by culture result, such as culture-positive and target-negative controls. There were significantly higher IgG binding levels in culture-positive samples for all pathogens ($p < 0.05$, unpaired two-tailed t-tests with Welch's correction). Table 1 details the differences in pathogen-specific IgG binding levels across each pathogen as well as grouped with culture results.

We compared the levels of pathogen-specific IgG binding based on the infection sites, which were classified into five groups: respiratory (38%), surgical site (27%), bacteremia (25%), urinary tract (8%), and other infections (2%). Only the non-infected controls had significantly lower binding levels than all other categories (Brown–Forsythe and Welch ANOVA, $p < 0.0001$; see Supplementary Fig. S1 online).

IgG binding in recurrent same-pathogen infections

Among the 315 participants, a small post-hoc longitudinal subset (13/315 patients, 4.1%) had repeat positive cultures for the same pathogen at different time points. There was no overall change in the same pathogen-specific

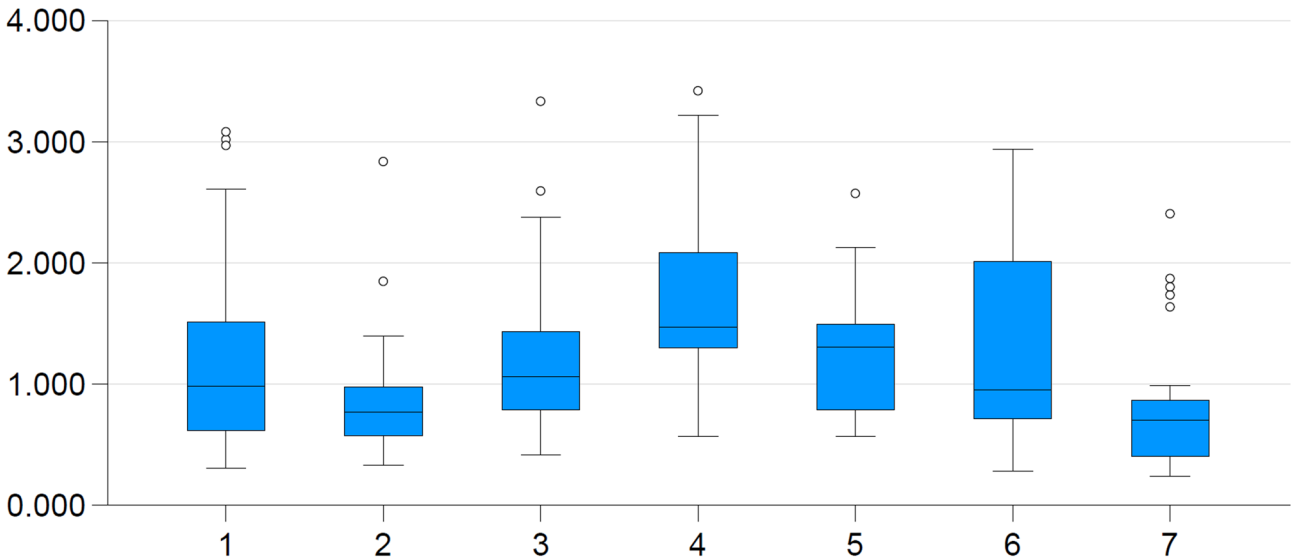

Fig. 1. IgG binding levels for each target pathogens. Box-and-whisker plots show the median (center line) and interquartile range (box); whiskers extend to 1.5×IQR (Tukey method). Individual observations (optical density measured at 405 nm) are overlaid; outliers beyond the whiskers are shown as open circles. Pathogens: (1) *Acinetobacter baumannii*; (2) *Klebsiella pneumoniae*; (3) *Escherichia coli*; (4) *Pseudomonas aeruginosa*; (5) *Enterococcus faecalis*; (6) *Enterococcus faecium; (7) Staphylococcus aureus*.

Pathogen	Culture-positive (*n*)	Mean [a] ± SD (OD)	Target-negative controls (*n*)	Mean ± SD (OD)	*p* value	Total (*n*)
A. baumannii	38	1.70 ± 0.58	47	0.66 ± 0.22	< 0.0001	85
K. pneumoniae	16	1.18 ± 0.54	24	0.65 ± 0.19	0.0015	40
E. coli	24	1.55 ± 0.65	26	0.86 ± 0.28	< 0.0001	50
P. aeruginosa	22	2.10 ± 0.72	21	1.27 ± 0.30	< 0.0001	43
E. faecalis	16	1.49 ± 0.46	12	0.88 ± 0.27	0.0005	28
E. faecium	14	2.11 ± 0.52	23	0.74 ± 0.23	< 0.0001	37
S. aureus	7	1.59 ± 0.56	25	0.59 ± 0.22	0.0029	32

Table 1. IgG binding levels for target pathogens by culture results. [a]Pathogen-specific IgG binding level as mean ± SD (standard deviation) OD (optical density, measured at 405 nm); Controls: Cases with negative culture results for the target pathogen; p-values by unpaired two-tailed t-test with Welch's correction; $p < 0.05$ significant.

IgG binding levels between the two infection episodes (Wilcoxon matched pairs signed rank test, $p = 0.892$); however, a moderate positive correlation was observed (Pearson's $r = 0.587$; $p = 0.034$). Only two patients in this subset reverted to seronegativity on days 3 and 9 after the initial culture (see Supplementary Fig. S2 online).

Impact of pathogen diversity on IgG binding efficacy

We explored the impact of pathogen diversity by comparing IgG binding levels among patients with different target pathogens. Out of the cohort, 47% exhibited growth of a single target pathogen, whereas 53% presented with infections from multiple pathogens. Our analysis revealed no significant differences in pathogen-specific IgG binding levels between these groups (Welch-corrected unpaired t test,$p = 0.962$).

We compared the pathogen-specific IgG binding levels in patients with simultaneous growth of two different targeted pathogens while excluding data from any third simultaneous pathogen to maintain data consistency. While the type of pathogen significantly impacted specific IgG binding levels (two-way ANOVA, $p = 0.037$), the presence of multiple target pathogens did not alter the pathogen-specific IgG binding levels ($p = 0.470$).

Diagnostic accuracy of the IgG binding assay

The optimized threshold value for the combined IgG binding assay was determined to be 1.009, based on ROC curve analysis using culture results as the reference standard. At this cutoff, the test was classified as an excellent A-category test with an ROC area of 0.910 (95% CI: 0.878 – 0.944, $p < 0.0001$). The analysis demonstrated high accuracy (83.2%), with a sensitivity of 85.4% and a specificity of 81.4%.

In addition to the global threshold, pathogen-specific OD cutoffs were calculated using Youden's index (sensitivity + specificity – 1) to optimize the performance of each individual assay. These cutoffs, determined via ROC analysis, represent the OD thresholds that best differentiate true positive from true negative results based on culture reference. ODs above a pathogen's cutoff were interpreted as positive IgG binding, indicating active or recent infection, whereas ODs below the cutoff indicated a negative result.

Among the pathogens tested, the *A. baumannii* IgG binding assay demonstrated the highest diagnostic accuracy (94.1%), with 94.7% sensitivity, 93.6% specificity, and an AUC of 0.975, followed by *S. aureus* (93.7%). The highest AUC was observed for *E. faecium* (0.990) (Table 2). Figure 2 displays the ROC curves of each target pathogen.

Categorical analysis and test reliability

We classified IgG binding OD values as positive or negative using pathogen-specific cutoffs derived from ROC curve analysis. Out of 315 samples, 132 (42.0%) were IgG-positive and 183 (58.0%) were IgG-negative for target infections. Of the 132 IgG-positive cases, 117 (88%, PPV) correlated with culture-positive results, whereas 15

Pathogens	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)	AUC	Cutoff (OD)
A. baumannii	94.7	93.6	94.1	92.3	95.6	0.975	1.041
K. pneumoniae	68.7	91.6	82.5	84.6	81.4	0.878	0.955
E. coli	83.3	76.9	80.0	76.9	83.3	0.842	1.050
P. aeruginosa	81.8	95.2	88.4	94.7	83.2	0.863	1.534
E. faecalis	81.3	83.3	82.1	86.7	76.9	0.875	1.266
E. faecium	71.4	87.0	81.1	76.9	83.3	0.990	1.327
S. aureus	85.7	93.7	93.7	85.7	96.0	0.954	0.918
Total	**85.4**	**81.4**	**83.2**	**78.0**	**87.8**	**0.910**	**1.009**

Table 2. Diagnostic test performance and optimal cutoffs of IgG binding assay. PPV: positive predictive value; NPV: negative predictive value; AUC: area under the Receiver Operating Characteristic (ROC) curve; OD (optical density, measured at 405 nm).

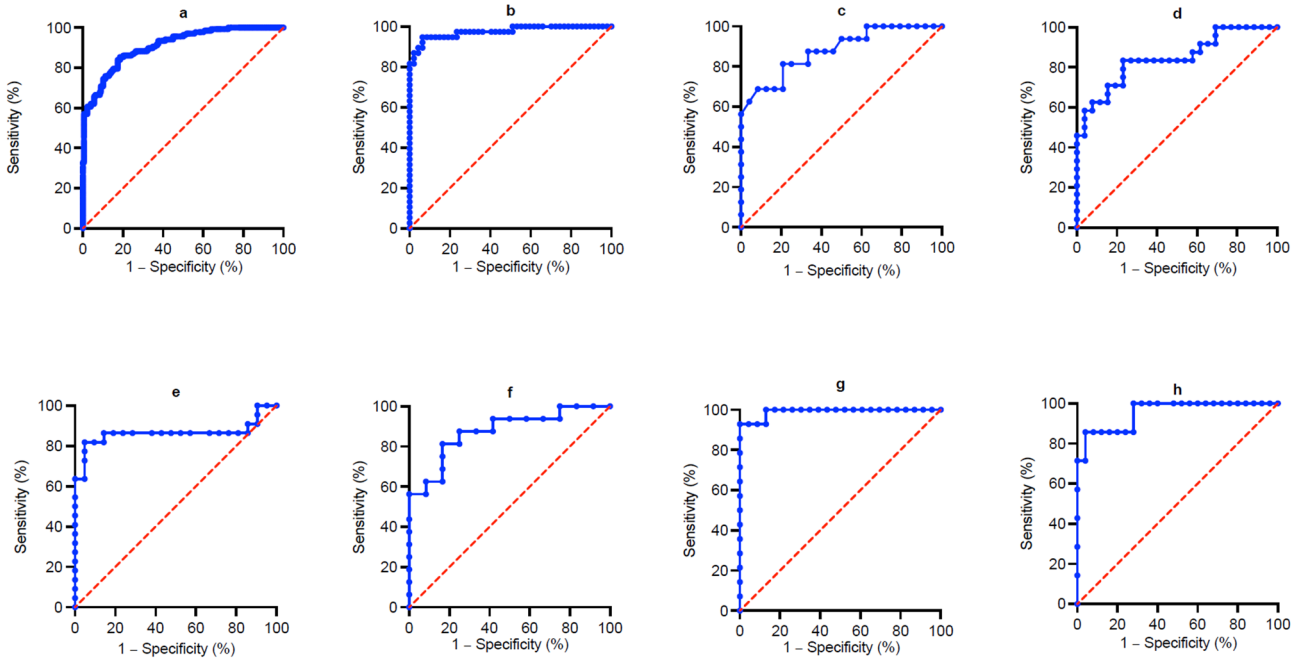

Fig. 2. Receiver Operating Characteristic (ROC) curves for IgG binding levels across the targeted pathogens. Panels: (**a**) All pathogens combined; (**b**) *Acinetobacter baumannii*; (**c**) *Klebsiella pneumoniae*; (**d**) *Escherichia coli*; (**e**) *Pseudomonas aeruginosa*; (**f**) *Enterococcus faecalis*; (**g**) *Enterococcus faecium; (h) Staphylococcus aureus.*

did not. Similarly,, 163 (89%, NPV) of the 183 IgG-negative results matched the culture-negative for the target pathogens, though 20 showed positive culture results. The contingency table for the categorical agreement analysis is provided in Supplementary Table S1 online.

The classification of pathogen-specific IgG assay demonstrated a strong correlation with culture results, achieving a categorical agreement of 88.9% and a Kappa statistic of 0.773, indicating substantial agreement. The odds ratio of 63.57 underscores the assay's strong predictive value for detecting true infections (Fisher's exact, 95% CI: 31.48–122.4, $p < 0.0001$, two-sided).

All 20 IgG-negative/culture-positive discordant cases had at least one target pathogen growth, but only 4 of these (Cases 1-4) were monomicrobial infections *with Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Acinetobacter baumannii.* The other 16 discordant cases involved polymicrobial growth: seven (Cases 5 -11) involved polymicrobial infections with two or more target pathogens; in these instances, serology was positive for some targets while co-isolates remained IgG-negative, and four also had non-target organisms. The remaining nine discordant cases (Cases 12 -20) were likewise polymicrobial but featured only one target pathogen alongside non-target organisms, most commonly *Proteus mirabilis*, *Candida spp.*, *Serratia marcescens*, and *Corynebacterium striatum* (see Supplementary Table S2 online).

The second discordant group, the 15 IgG-positive/culture-negative cases, was clinically milder. Only five of these were IgG-positive for a single pathogen (2.8% [5/178] of culture-negative cases). The other ten were drawn during empirical antibiotic treatment, and in three of those, the same pathogen grew on repeat culture within 72 h. The 20 IgG-negative / culture-positive group had increased ICU mortality (55% vs. 20%; $p = 0.046$), higher inflammation (CRP median 165 mg/L vs. 86 mg/L ; $p = 0.032$), and needed more frequent mechanical ventilation (90% vs. 40%; $p = 0.0028$) compared with the 15 IgG-positive / culture-negative group (see Supplementary Table S3 online).

Discussion

In this prospective observational cohort study conducted in two ICUs, we included patients who developed infections with one of seven target pathogens and analyzed their serum samples to compare the performance of an ELISA-based IgG binding assay with the standard culture method for diagnosing HAIs. Although culture remains the reference standard, its utility in ICU settings is limited by false negatives in antibiotic-treated patients and false positives arising from colonization[17,18].

IgG binding accurately identifies infections, aligning with established literature[19,20], and ELISA demonstrates high specificity and sensitivity in identifying pathogens in polymicrobial environments[21,22]. Despite the high incidence of secondary pathogens in our cohort, no significant impact on target pathogen-specific IgG binding levels was observed, demonstrating the assay's accuracy without cross-reactivity. IgG effectively targets pathogens in both single and polymicrobial infections and plays a fundamental and stable role in the body's defense mechanisms, regardless of the infection's complexity. This highlights the dependable and wide-ranging effectiveness of IgG in adaptive immunity[23,24]. In our study, we found no significant differences in IgG binding levels between single and multiple target pathogen infections; this suggests that pathogen-specific IgG binding

levels were consistent, regardless of the target pathogen complexity. There was a small subset of patients (4.1% of the cohort) with positive cultures of the same pathogen, and we observed no overall change in IgG-binding levels between the infection episodes.

Only two individuals reverted to seronegativity on day 3 and day 9, respectively. The rapid decline observed in these two cases may reflect the immediate but short-lived nature of IgG–FcγR interactions, which prioritize rapid response over long-term persistence typical of adaptive immunity[25,26].

Studies broadly emphasize the role of IgG in immune responses without specific references to the infection locations[27,28], but they are more influenced by the severity of the disease, supporting the utility of IgG assays across different types of infections[29,30]. In our study, there were also no significant differences in IgG binding levels between sample types or infection sites, indicating that the IgG binding assay can be used for any type of infection without losing sensitivity or specificity.

Our previous study[31], which included immunosuppressed individuals, demonstrated minimal impact on the effectiveness of the IgG binding assay, addressing concerns about the variability in immune response, especially in immunocompromised patients. Out of the 79 patients in that study, only two exhibited a low phagocytic index. This indicates that the assay's diagnostic accuracy remains reliable across diverse patient populations, including those with compromised immune responses.

Previous research supports our findings on the utility of IgG binding in infection management. Goh et al. observed a 48% increase in *Salmonella typhimurium* phagocytosis linked to IgG3 isotypes[32]. Similarly, Dunnen et al. reported that IgG binding boosts Th17 cell production in response to *S. aureus* and *K. pneumoniae*[23], and another study highlighted opsonins' role in enhancing *S. epidermidis* phagocytosis[33], thus improving pathogen recognition and ingestion by phagocytes. Bardardottir et al. demonstrated increased binding post-vaccination, indicating that immune activation enhances IgG levels and pathogen targeting[34]. Our results extend these observations by demonstrating high sensitivity (85.4%) and specificity (81.4%) in distinguishing culture-positive from target-negative cases through IgG binding. These findings align with the performance metrics of established diagnostic tests[35,36].

Our ROC-derived cutoffs revealed wide interspecies differences in assay accuracy. *P. aeruginosa* antigens (largely lipid A variants) demonstrated the highest median OD but a broader distribution (AUC 0.863), consistent with extensive lipopolysaccharide heterogeneity among clinical isolates[37,38]. By contrast, *A. baumannii*'s conserved outer-membrane proteins may be clustered tightly (AUC 0.975), driving superior sensitivity (94.7%) and specificity (93.6%)[39]. Species with known immune-evasion features, *K. pneumoniae* (polysaccharide capsule)[40,41] and *S. aureus* (protein A), showed lower sensitivities (68.8% and 85.7%, respectively) despite high specificity, reflecting masked or variable epitope exposure[42,43]. *E. coli* and *E. faecalis*, with readily accessible surface antigens, delivered intermediate performance (AUC 0.842 and 0.875)[44,45].

In our cohort, despite excellent overall performance (AUC 0.91), we observed 20 IgG-negative/culture-positive and 15 IgG-positive/culture-negative discordant cases. Only 4/20 cases (2.9% of culture-positives) were IgG-negative with single target-pathogen growths, and 5/15 cases (2.8% of culture-negatives) were IgG-positive without antibiotic pressure; these likely represent the true upper limit for false negatives and false positives, respectively. Sixteen of the IgG-negative/culture-positive discordances involved polymicrobial infections. In seven mixed-target cases (5–11), serology was positive for some targets while co-isolates remained IgG-negative. Four of these seven also had non-target organisms (see Supplementary Table S1 online). Such pathogen-specific immunodominance of one target over another[46–48], or sepsis-associated immunoparalysis as a profound systemic inflammation delaying IgG class switching, has been well documented in other settings[49,50]. In the remaining 13/20 (65%) cases, we recovered non-target organisms (e.g., *Proteus mirabilis*, *Serratia marcescens*, and *Corynebacterium striatum*) alongside one target pathogen. These patterns suggest that traditional culture may recover colonizers under heavy antibiotic pressure rather than the principal immunogenic pathogen[51,52].

The 15 IgG-positive/culture-negative cases further illustrated this host-pathogen disconnect: 10/15 (67%) were drawn during empirical antibiotic therapy; antibiotics likely suppressed culture growth, even though early IgG seroconversion had occurred, as confirmed by repeat cultures in 3/10 within 72 h. Empirical antibiotic administration prior to sample collection is well known to suppress culture positivity[53,54].

The IgG-negative/culture-positive subgroup had markedly worse clinical parameters (median ICU stay 26.8 vs. 6.1 days; CRP 165 vs. 86 mg/L; 90% vs. 40% on ventilators; 55% vs. 20% mortality; all $p < 0.05$), compared with the IgG-positive/culture-negative cases. This suggests that their extreme inflammatory state and immune exhaustion may prevent a detectable IgG, explaining why they deviate from the overall mono- vs. polymicrobial patterns[55]. Together, these data underscore that culture and serology are complementary: culture confirms viability, whereas IgG reveals host engagement, and integrating both, with clinical context, can optimize HAI diagnosis in the ICU.

Implementing the IgG binding assay in ICUs can cut pathogen identification from days to roughly 4 h, enabling earlier, more informed antibiotic choices[56]. Although our IgG assay does not provide antimicrobial-susceptibility results, studies of other rapid diagnostics in bloodstream infections have demonstrated that quick turnaround can shorten time to targeted therapy and reduce broad-spectrum empiric use[57].

Multiplex PCR panels identify pathogens and resistance markers in 2 h, and MALDI-TOF MS provides species ID in < 1 h post-isolation, but both require specialized instruments and do not assess host response[3,5]. EUCAST RAST uses standard blood-culture incubators and disk readers to provide resistance profiles in 4 h[4]. In contrast, the ELISA-based pathogen-specific IgG binding assay runs on common 96-well readers and delivers pathogen-specific host-response data in 4 h[14]. Crucially, by measuring pathogen-specific IgG binding levels, it reports on the host's immune engagement, enabling detection of true infections even under antibiotic therapy, early in disease progression, or when culture and molecular tests give false positives due to colonization or residual non-viable DNA[12,58].

This study successfully optimized an IgG-binding ELISA targeting a specific panel of seven critical ICU pathogens, achieving a swift turnaround while maintaining high sensitivity and specificity. The high performance metrics reflect both the controlled nature of our study cohort and the targeted optimization of the assay. The collection and analysis of blind samples effectively reduced bias, while advanced statistical methods reinforced the strength of our findings. However, our study was conducted in a single geographic setting with a predefined panel of seven pathogens, so findings may not generalize to other institutions or non-target organisms. The use of standard culture as the sole reference point limited accurate determination of true-positive and true-negative rates, since culture often fails to detect infections in patients receiving antibiotic therapy or to distinguish colonization from invasive disease. In our small paired subset, the clinically defined follow-up times precluded mapping of seroreversion kinetics. Finally, while our assay rapidly confirms pathogen presence, it does not directly provide data on antimicrobial susceptibility. Therefore, future work should focus on (1) longitudinal sampling to map seroconversion kinetics; (2) expanding antigen libraries to cover strain heterogeneity; and (3) integrating rapid IgG profiling with molecular diagnostics and fast AST platforms to deliver a comprehensive, one-stop workflow for pathogen identification, host-response assessment, and susceptibility testing.

In conclusion, our pathogen-specific IgG binding assay offers a rapid (≈4 h) and accurate complement to culture for diagnosing HAIs in ICU patients. By enabling earlier, targeted therapy, it has the potential to improve clinical outcomes, shorten lengths of stay, reduce unnecessary antibiotic usage, and ultimately lower healthcare costs.

Data availability

The data that support the findings of this study are not openly available due to sensitivity reasons and are available from the corresponding author upon reasonable request. The data are stored in controlled-access storage systems by the corresponding author.

Received: 2 September 2024; Accepted: 25 November 2025
Published online: 21 February 2026

References

1. World Health Organization. *Global Report on Infection Prevention and control. Geneva* (World Health Organization, 2022). https://www.who.int/publications/i/item/9789240103986
2. Rentschler, S., Kaiser, L. & Deigner, H. P. Emerging options for the diagnosis of bacterial infections and the characterization of antimicrobial resistance. *Int. J. Mol. Sci.* **22**, 456. https://doi.org/10.3390/ijms22010456 (2021).
3. Yu, J. et al. Prediction of methicillin-resistant *Staphylococcus aureus* and carbapenem-resistant *Klebsiella pneumoniae* from flagged blood cultures by combining rapid sepsityper MALDI-TOF mass spectrometry with machine learning. *Int. J. Antimicrob. Agents.* **62**, 106994. https://doi.org/10.1016/j.ijantimicag.2023.106994 (2023).
4. Berinson, B. et al. EUCAST rapid antimicrobial susceptibility testing (RAST): analytical performance and impact on patient management. *J. Antimicrob. Chemother.* **76**, 1332–1338. https://doi.org/10.1093/jac/dkab026 (2021).
5. Tóth, Z. et al. Application of biofire filmarray joint infection panel for rapid identification of aetiology in a necrotizing fasciitis case. *Diagnostics (Basel).* **15**, 58. https://doi.org/10.3390/diagnostics15010058 (2024).
6. Morel, C. et al. Ensuring innovation in diagnostics for bacterial infection: Implications for policy [Internet]. Copenhagen (Denmark): European Observatory on Health Systems and Policies (2016). https://www.ncbi.nlm.nih.gov/books/NBK447319
7. Li, S. et al. A clinical diagnostic study: Fibulin-2 is a novel promising biomarker for predicting infection. *Infect. Dis. Ther.* **11**, 1057–1073. https://doi.org/10.1007/s40121-022-00622-y (2022).
8. Arienzo, A. et al. A new point-of-care test for the rapid antimicrobial susceptibility assessment of uropathogens. *PLoS One.* **18**, e0284746. https://doi.org/10.1371/journal.pone.0284746 (2023).
9. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* **399**, 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0 (2022).
10. Winkelstein, J. A. Opsonins: Their function, significance, identity, and clinical implications. *J. Pediatr.* **82**, 747–753. https://doi.org/10.1016/s0022-3476(73)80062-9 (1973).
11. Thau, L., Asuka, E. & Mahajan, K. Physiology Opsonization. In *StatPearls* (2024). https://www.ncbi.nlm.nih.gov/books/NBK304809/
12. Xu, N., Wang, W., Chen, F., Li, W. & Wang, G. ELISA is superior to bacterial culture and agglutination test in the diagnosis of brucellosis in an endemic area in China. *BMC Infect. Dis.* **20** https://doi.org/10.1186/s12879-019-4729-1 (2020).
13. Murray, P. R., Rosenthal, K. S. & Pfaller, M. A. *Medical Microbiology* 9th edn (Elsevier, 2021).
14. Elder, B. L., Boraker, D. K. & Fives-Taylor, P. M. Whole-bacterial cell enzyme-linked immunosorbent assay for *Streptococcus Sanguis* fimbrial antigens. *J. Clin. Microbiol.* **16**, 141–144. https://doi.org/10.1128/jcm.16.1.141-144.1982 (1982).
15. GraphPad, S. GraphPad Prism version 10.5.0 (673). GraphPad Software (2025). https://www.graphpad.com/scientific-software/prism/
16. Zar, J. H. *Biostatistical Analysis* 5th edn (Prentice Hall, 2009).
17. Rawson, T. M., Antcliffe, D. B., Wilson, R. C., Abdolrasouli, A. & Moore, L. S. P. Management of bacterial and fungal infections in the ICU: Diagnosis, treatment, and prevention recommendations. *Infect. Drug Resist.* **16**, 2709–2726. https://doi.org/10.2147/IDR.S390946 (2023).
18. Fernández, L. et al. Developing diagnostic and therapeutic approaches to bacterial infections for a new era: implications of globalization. *Antibiot. (Basel).* **9**, 916. https://doi.org/10.3390/antibiotics9120916 (2020).
19. Rakita, R. M. et al. Specific antibody promotes binding and PMN-mediated killing of phagocytosis-resistant *Enterococcus faecium*. *FEMS Immunol. Med. Microbiol.* **28**, 291–299. https://doi.org/10.1111/j.1574-695X.2000.tb01489.x (2000).
20. Boulouis, C. et al. Binding-enhanced antigen presentation by MR1 activates rapid polyfunctional MAIT cell responses acting as an effector arm of humoral antibacterial immunity. *J. Immunol.* **205**, 67–77. https://doi.org/10.4049/jimmunol.2000003 (2020).
21. Cassedy, A., Parle-McDermott, A. & O'Kennedy, R. Virus detection: A review of the current and emerging molecular and immunological methods. *Front. Mol. Biosci.* **8**, 637559. https://doi.org/10.3389/fmolb.2021.637559 (2021).
22. Yow, K. S., Aik, J., Tan, E. Y., Ng, L. C. & Lai, Y. L. Rapid diagnostic tests for the detection of recent dengue infections: an evaluation of six kits on clinical specimens. *PLoS One.* **16**, e0249602. https://doi.org/10.1371/journal.pone.0249602 (2021).
23. den Dunnen, J. et al. IgG binding of bacteria promotes Th17 responses via synergy between TLRs and FcγRIIa in human dendritic cells. *Blood* **120**, 112–121. https://doi.org/10.1182/blood-2011-12-399931 (2012).
24. Lu, L. L., Suscovich, T. J., Fortune, S. M. & Alter, G. Beyond binding: antibody effector functions in infectious diseases. *Nat. Rev. Immunol.* **18**, 46–61. https://doi.org/10.1038/nri.2017.106 (2018).

25. Chen, X. et al. FcγR-binding is an important functional attribute for immune checkpoint antibodies in cancer immunotherapy. *Front. Immunol.* **10**, 292. https://doi.org/10.3389/fimmu.2019.00292 (2019).
26. Fransen, M. F. et al. A restricted role for FcγR in the regulation of adaptive immunity. *J. Immunol.* **200**, 2615–2626. https://doi.org/10.4049/jimmunol.1700429 (2018).
27. Muruganandah, V. & Kupz, A. Immune responses to bacterial lung infections and their implications for vaccination. *Int. Immunol.* **34**, 231–248. https://doi.org/10.1093/intimm/dxab109 (2022).
28. Geyer, C. E., Mes, L., Newling, M., den Dunnen, J. & Hoepel, W. Physiological and pathological inflammation induced by antibodies and pentraxins. *Cells* **10**, 1175. https://doi.org/10.3390/cells10051175 (2021).
29. Rijkers, G. et al. Differences in antibody kinetics and functionality between severe and mild severe acute respiratory syndrome coronavirus 2 infections. *J. Infect. Dis.* **222**, 1265–1269. https://doi.org/10.1093/infdis/jiaa463 (2020).
30. Hassan, A. & Blanchard, N. Microbial (co)infections: powerful immune influencers. *PLOS Pathog.* **18**, e1010212. https://doi.org/10.1371/journal.ppat.1010212 (2022).
31. Karakullukçu, A., Akker, M., Kuşkucu, M. A., Dikmen, Y. & Aygün, G. Diagnostic role of opsonic activity in *Acinetobacter baumannii* ventilator-associated pneumonia. *Diagn. Microbiol. Infect. Dis.* **109**, 116262. https://doi.org/10.1016/j.diagmicrobio.2024.116262 (2024).
32. Goh, Y. S. et al. Human IgG isotypes and activating Fcγ receptors in the interaction of *Salmonella enterica* serovar typhimurium with phagocytic cells. *Immunology* **133**, 74–83. https://doi.org/10.1111/j.1365-2567.2011.03411.x (2011).
33. Katzenmeyer, K. N., Szott, L. M. & Bryers, J. D. Artificial Opsonin enhances bacterial phagocytosis, oxidative burst and chemokine production by human neutrophils. *Pathog Dis.* **75**, ftx075. https://doi.org/10.1093/femspd/ftx075 (2017).
34. Bardardottir, E., Jonsson, S., Jonsdottir, I., Sigfusson, A. & Valdimarsson, H. IgG subclass response and opsonization of Streptococcus pneumoniae after vaccination of healthy adults. *J. Infect. Dis.* **162**, 482–488. https://doi.org/10.1093/infdis/162.2.482 (1990).
35. van de Ven, N. L. M. et al. Point-of-care neutrophil CD64 as a rule-in diagnostic test for bacterial infections in the emergency department. *BMC Emerg. Med.* **23**, 28. https://doi.org/10.1186/s12873-023-00800-2 (2023).
36. Chong, Z. L. et al. Diagnostic accuracy and utility of three dengue diagnostic tests for the diagnosis of acute dengue infection in Malaysia. *BMC Infect. Dis.* **20**, 210. https://doi.org/10.1186/s12879-020-4911-5 (2020).
37. Constantino-Teles, P., Jouault, A., Touqui, L. & Saliba, A. M. Role of host and bacterial lipids in *Pseudomonas aeruginosa* respiratory infections. *Front. Immunol.* **13**, 931027. https://doi.org/10.3389/fimmu.2022.931027 (2022).
38. Stanislavsky, E. S. & Lam, J. S. *Pseudomonas aeruginosa* antigens as potential vaccines. *FEMS Microbiol. Rev.* **21**, 243–277. https://doi.org/10.1111/j.1574-6976.1997.tb00353.x (1997).
39. Jeffreys, S. et al. Insights into *Acinetobacter baumannii* protective immunity. *Front. Immunol.* **13**, 1070424. https://doi.org/10.3389/fimmu.2022.1070424 (2022).
40. Huang, X. et al. Capsule type defines the capability of *Klebsiella pneumoniae* in evading Kupffer cell capture in the liver. *PLoS Pathog.* **18**, e1010693. https://doi.org/10.1371/journal.ppat.1010693 (2022).
41. Liang, Z. et al. Host defense against the infection of *Klebsiella pneumoniae*: new strategy to kill the bacterium in the era of antibiotics? *Front. Cell. Infect. Microbiol.* **12**, 1050396. https://doi.org/10.3389/fcimb.2022.1050396 (2022).
42. van Kessel, K. P. M., Bestebroer, J. & van Strijp, J. A. G. Neutrophil-mediated phagocytosis of *Staphylococcus aureus*. *Front. Immunol.* **5**, 467. https://doi.org/10.3389/fimmu.2014.00467 (2014).
43. McDowell, G., Grov, A. & Oeding, P. Reaction of Staphylococcal protein A with rabbit Immunoglobulins. *Acta Pathol. Microbiol. Scand. B Microbiol. Immunol.* **79B**, 794–800. https://doi.org/10.1111/j.1699-0463.1971.tb00113.x (1971).
44. Mishra, R. P. N. et al. Structural and immunological characterization of *E. coli* derived Recombinant CRM197 protein used as carrier in conjugate vaccines. *Biosci. Rep.* **38**, BSR20180238. https://doi.org/10.1042/BSR20180238 (2018).
45. Katzenmeyer, K. N. & Bryers, J. D. Multivalent artificial Opsonin for the recognition and phagocytosis of Gram-positive bacteria by human phagocytes. *Biomaterials* **32**, 4042–4051. https://doi.org/10.1016/j.biomaterials.2011.02.007 (2011).
46. Ferreira, R. B. R. & Antunes, L. C. M. Sal-Man, N. Pathogen-pathogen interactions during co-infections. *ISME J.* **19**, wraf104. https://doi.org/10.1093/ismejo/wraf104 (2025).
47. Salie, M. T. et al. Serum immune responses to group A Streptococcal antigens following pharyngeal acquisitions among children in cape Town, South Africa. *mSphere* **8**, e0011323 (2023).
48. Racine, R. et al. Impaired germinal center responses and suppression of local IgG production during intracellular bacterial infection. *J. Immunol.* **184**, 5085–5093. https://doi.org/10.4049/jimmunol.0902710 (2010).
49. Cao, M., Wang, G. & Xie, J. Immune dysregulation in sepsis: experiences, lessons and perspectives. *Cell. Death Discov.* **9**, 465. https://doi.org/10.1038/s41420-023-01766-7 (2023).
50. Frattari, A. et al. Analysis of peripheral blood lymphocyte subsets in critical patients at ICU admission: A preliminary investigation of their role in the prediction of sepsis during ICU stay. *Int. J. Immunopathol. Pharmacol.* **32**, 2058738418792310. https://doi.org/10.1177/2058738418792310 (2018).
51. Calvo, M., Stefani, S. & Migliorisi, G. Bacterial infections in intensive care units: epidemiological and Microbiological aspects. *Antibiot. (Basel).* **13**, 238. https://doi.org/10.3390/antibiotics13030238 (2024).
52. Vincent, J. et al. International study of the prevalence and outcomes of infection in intensive care units. *JAMA* **302**, 2323–2329. https://doi.org/10.1001/jama.2009.1754 (2009).
53. Harris, A. M. et al. Influence of antibiotics on the detection of bacteria by culture-based and culture-independent diagnostic tests in patients hospitalized with Community-Acquired pneumonia. *Open. Forum Infect. Dis.* **4**, ofx014. https://doi.org/10.1093/ofid/ofx014 (2017).
54. Scheer, C. S. et al. Impact of antibiotic administration on blood culture positivity at the beginning of sepsis: a prospective clinical cohort study. *Clin. Microbiol. Infect.* **25**, 326–331. https://doi.org/10.1016/j.cmi.2018.05.016 (2019).
55. Liu, Z. et al. Immunosenescence: molecular mechanisms and diseases. *Signal Transduct. Target. Ther.* **8** https://doi.org/10.1038/s41392-023-01451-2 (2023).
56. Barlam, T. F. et al. Implementing an antibiotic stewardship program: guidelines by the infectious diseases society of America and the society for healthcare epidemiology of America. *Clin. Infect. Dis.* **62**, e51–e77. https://doi.org/10.1093/cid/ciw118 (2016).
57. Banerjee, R. et al. Randomized trial of rapid multiplex polymerase chain Reaction-Based blood culture identification and susceptibility testing. *Clin. Infect. Dis.* **61**, 1071–1080. https://doi.org/10.1093/cid/civ447 (2015).
58. Sheldon, I. M. Detection of pathogens in blood for diagnosis of sepsis and beyond. *eBioMedicine* **9**, 13–14. https://doi.org/10.1016/j.ebiom.2016.06.030 (2016).

Acknowledgements

The authors would like to thank Prof. Dr. Can Günay from Cerrahpaşa University Hospital for statistical assistance and Mürsel Baydemir from Bogazici University for helping with the acquisition of data.

Author contributions

AK has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the study's conception and design. Material preparation

and analysis were performed by AK, data collection was performed by MA, and visualization was performed by MAK. The first draft of the manuscript was written by AK. YD and GA provided critical revisions and supervision. All authors revised it critically for important intellectual content. All authors read and approved the final manuscript. All authors gave final approval for the version to be published.

Funding
This study was funded by the Turkish Scientific and Technological Research Council [Project Number: 20170294 to Asiye Karakullukçu].

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-30459-7.

Correspondence and requests for materials should be addressed to A.K.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.