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Time to Positivity of Blood Culture in an Automated Bact/Alert 3D System and Its Association with Procalcitonin Levels and Organism Type: A Retrospective Observational Study

Sandhiya R
SRM Institute of Science and Technology
Leela K V
SRM Institute of Science and Technology
Jayaprakash T
jpmicro74@gmail.com

SRM Institute of Science and Technology
Rahul H L
SRM Institute of Science and Technology

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Abstract

Background

Bloodstream infections (BSIs) are life-threatening conditions frequently encountered in critical care, where early identification of infection and timely initiation of appropriate antimicrobial therapy are essential for reducing morbidity and mortality. Although blood culture remains the reference method for microbiological conformation, automated blood culture systems provide rapid and standardized detection of microbial growth. Time to positivity (TTP) may provide additional information regarding the infecting organism and microbial burden. While procalcitonin (PCT) is widely used as an adjunctive biomarker in the assessment of suspected bacterial infection and sepsis. In this study to determine the TTP of clinically significant blood cultures processed using the automated Bact/Alert 3D system and evaluate its relationship with serum procalcitonin levels. The study also assessed organism-specific differences in TTP and relevant demographic associations.

Methods

A retrospective observational study was conducted in the Department of Microbiology, SRM Medical College Hospital and Research Centre, Kattankulathur, Tamil Nadu, from September 2025 to August 2026. All blood culture samples received during the study period were included. Samples were collected under strict aseptic conditions and processed using the automated Bact/Alert 3D system. TTP was recorded in hours. Available serum PCT values were correlated with TTP, and organism-specific differences were statistically analyzed.

Results

Of the total suspected blood stream infection culture samples analyzed, 8,726(90.2%) showed no growth after five days, 514(5.3%) yielded skin commensals, and 434(4.5%) yielded clinically significant organisms. A valid TTP was obtained for 259 positive cultures. The mean TTP was 14.36 ± 13.67hours, with a median of 9.21 hours (IQR 6.00–16.28). Overall, 62.9% of cultures became positive within 12 hours, 82.6% within 24 hours, and 95.8% within 48% hours. Gram-negative organisms predominated (59.1%), followed by Gram positive organisms (30.9%) and Candida species (8.1%). Candida showed the longest median TTP (32.01 h; $p < 0.001$). PCT showed a significant inverse correlation with TTP ($p = -0.270$, $p < 0.001$).

Conclusion

TTP varied significantly by organism and showed an inverse correlation with PCT. Combining TTP and PCT may support early risk assessment and clinical management of sepsis in critical care settings.

INTRODUCTION

Bloodstream infection is a serious clinical condition that can progress rapidly to sepsis, septic shock, multiorgan dysfunction, and death [1]. Early recognition of bloodstream infection and timely initiation of appropriate antimicrobial therapy are therefore essential components of patient management [2].

Blood culture remains an important reference method for the microbiological diagnosis of bloodstream infection because it permits recovery and subsequent identification of the causative organism [3]. The introduction of automated bloodculture systems has substantially improved the detection of microbial growth and reduced the time required for laboratory notification of positive cultures. The Bact/Alert 3D system uses automated colorimetric detection of microbial growth and provides continuous monitoring of blood culture bottles [4].

Time to positivity is the period between initiation of incubation and automated detection of microbial growth [5]. TTP can vary according to the organism, microbial concentration, blood volume inoculated, prior antimicrobial exposure, culture media, and other pre-analytical and analytical factors [6, 7, 8, 9]. Several studies have demonstrated that TTP may provide useful information about the type of pathogen and may have potential clinical implications in bloodstream infection [10, 11].

Procalcitonin is another important laboratory marker investigated in patients with suspected bacterial infection and bloodstream infection. Studies have demonstrated that PCT may provide useful diagnostic information, particularly in relation to bacteremia and Gram-negative bloodstream infection. However, PCT should be considered an adjunctive test rather than a replacement for blood culture [12, 13].

The simultaneous assessment of TTP and PCT may therefore provide complementary microbiological and serological information. The present study was undertaken to determine TTP among positive blood cultures processed using the Bact/Alert 3D system and to investigate its relationship with serum PCT levels.

OBJECTIVES

Primary objective

To determine the time to positivity of clinically significant blood cultures processed using the automated Bact/Alert 3D system and evaluate its relationship with serum procalcitonin levels.

Secondary objectives

1. To determine the distribution of TTP among different isolated organisms.
2. To describe the distribution of bloodstream pathogens detected during the study period.

MATERIALS AND METHODS

Study design

The study was designed as a retrospective observational clinical laboratory-based study. Patient confidentiality was maintained throughout the study. Identifying information was not required for statistical analysis and removed from the research datasets used for analysis and publication. Institute Ethics Committee approval was obtained (8715/IEC/2023).

Study setting

The study was conducted in the Department of Microbiology, SRM Medical College Hospital and Research Centre, Kattankulathur-603203, Tamil Nadu, INDIA.

Study period

The study was conducted from September 2025 to August 2026.

Study population

Inclusion

All hospitalized patients with suspected bloodstream infection or sepsis whose blood samples were submitted for culture during the study period were included in the study.

Exclusion

No fever and walking patient

Blood Culture Sample Collection Procedure

Blood was collected under strict aseptic conditions. The skin was disinfected using a combination of chlorhexidine and alcohol, using a minor-surgical-procedure approach with concentric circles from the centre outward. The required volume of blood was collected according to the patient's age and culture requirements. Care was taken to avoid contact of the needle with potentially contaminated surface before inoculation. The flip cap of the blood culture bottle was opened and the bottle septum was cleaned using an alcohol swab before inoculation.

We use the blood collecting volume as Adults 8–10 ml, Children up to 4 ml. Paediatric patients were sampled using Paediatric blood culture bottles as appropriate. The inoculated bottles were transported to the Department of Microbiology. Blood culture bottle were not refrigerated in the event of a delay in transportation.

Basic Operating Procedure of Bact/Alert 3D system

The instrument worked in the colorimetric principle for the detection of bacterial growth by production of carbon dioxide of microorganism, which produces a change in the colorimetric sensor at the bottom of the culture bottle. The instrument detects these changes automatically during incubation. The Bact/Alert 3D system monitors culture bottles at regular intervals, enabling early detection of microbial growth.

Loading of blood culture bottles

The following workflow was followed:

1. The load bottles function was selected.
2. The bottle barcode was scanned
3. The accession number (microbiology number) was entered.
4. An available instrument drawer was selected
5. The bottle was loaded into a cell indicated as available by the instrument.
6. Additional bottles were loaded sequentially using the same procedure.
7. Instrument alarms, when encountered, were addressed according to the manufacturer's operating procedure.
8. After completion of loading, the appropriate confirmation function was selected to save the data.

Unloading positive bottles

Positive bottles were removed promptly after instrument notification.

1. The positive bottle unloading function was selected.
2. The drawer indicated by the instrument was opened.
3. Positive bottles were removed from the illuminated cells.
4. Bottles were handled individually and scanned as required.
5. Following removal, the corresponding instrument cell underwent its automated quality control cycle.
6. The unloading process was completed using the appropriate confirmation function.

Positive bottles were subsequently processed to identification and susceptibility testing procedure.

Unloading negative bottles

Negative bottles were removed after 5 days.

1. The negative bottle unloading function was selected.
2. The drawer indicated by the instrument was opened.
3. Negative bottles were removed from the indicated cells.
4. The corresponding cells underwent the automated QC process.
5. The unloading process was completed using the instrument confirmation function.

Review of bottle data

Bottle specific data and growth curves were reviewed and bottle identification associated instrument data were checked when required. Operational qualification and performance qualification of the Bact/Alert 3D system were performed at installation by the biomerieux application specialist. Performance quality control was performed using blood culture bottles inoculated with known ATCC reference organisms. Following instrument positivity, the sample was Gram stained, subculture and antibiotic sensitivity and organism identification processed. Lot to lot verification was performed using appropriate ATCC strains whenever a new blood culture bottle lot was introduced. These procedures were undertaken to ensure continued analytical performance and reliability of the automated blood culture system.

Potential interferences

The following factors were recognized as potential sources of interference or inaccurate results:

1. Contamination of specimens due to inadequate aseptic collection.
2. Improper blood collection technique.
3. Obstruction or improper reading of the barcode.

Appropriate specimen collection and bottle handling procedures were therefore followed to minimize pre analytical errors.

Principle and Calculation of Results

The Bcat/Alert 3D system detects microbial growth by monitoring carbon dioxide production in the culture bottle. Microbial metabolism changes in the colorimetric substrate located at the bottom of the bottle. The instrument sensor detects this colorimetric change and automatically flags the bottle as positive when the predefined detection criteria are met. The system monitors bottles at approximately 10 minutes intervals.

Time to Positivity

TTP was defined as: Time of automated positive detection – Time of initiation of incubation, and TTP was expressed in hours.

The relationship between TTP and PCT was assessed using spearman’s rank correlation. Differences in TTP among organism group were evaluated using the Kruskal-Wallis test.

RESULT

A total of 9,674 blood samples from patients with suspected bloodstream infections were collected over a 12-month period (September 2025 to August 2026), as shown in Figure-1.

Time to Positivity cohort

Total number of significant organism growth was 434(4.5%) among this 259 specimen TTP was recorded and analysed.

The distribution of TTP was:

- Mean: 14.36 ± 13.67 hours
- Median: 9.21 hours
- IQR: 6.00–16.28 hours
- Minimum: 1.92 hours
- Maximum: 65.28 hours

The majority of positive cultures became positive relatively early.

TTP interval	Number	Percentage
≤ 6 hours	67	25.90%
≤ 12 hours	163	62.90%
≤ 24 hours	214	82.60%
≤ 48 hours	248	95.80%
> 48 hours	11	4.20%

Table-1. Time to Positivity

In these results, more than four-fifths of cultures with documented TTP were detected within 24 hours (Table-1).

Distribution of Organisms

Among the 259 specimens with documented Time-to-positivity (TTP) results, Gram-negative organisms accounted for the largest proportion of isolates, as illustrated in Figure-2.

The most frequently recorded individual organisms included Escherichia coli, Klebsiella pneumoniae, MR-CoNS, Non-Fermenting Gram Negative Bacilli, Acinetobacter species, Streptococcus species, Salmonella species, and Staphylococcus aureus.

TTP according to Organism wise

The distribution of Time-to-Positivity (TTP) among the specific organisms was right- skewed, with most isolates becoming positive relatively early, while a smaller proportion exhibited substantially prolonged

TTP as shown in the Table-2.

Organism	Total number	Mean TTP (h)	Median TTP (h)	Range (h)
Escherichia coli	56	12.1	6.21	1.92–45.80
Klebsiella pneumoniae	33	6.49	5.01	1.92–34.32
CoNS	28	10.54	9.64	6.01–16.34
Acinetobacter baumannii	15	8.19	8.02	5.06–12.00
MR-CoNS	14	12.89	9.12	4.32–38.64
Non-fermenting Gram-negative bacilli	12	36.66	35.28	12.24–65.28
Enterococcus faecalis	10	16.71	11.17	2.01–46.80
Enterococcus sp.	8	11.12	13.34	1.92–19.24
Burkholderiacepacia	8	11.52	11.52	8.09–15.84
MRSA	7	8.05	6.07	5.04–13.01
Staphylococcus aureus	6	8.72	8.26	6.24–12.04
Streptococcus sp.	6	8.66	9.84	3.60–10.81
Candida spp.	5	46.42	52	28.08–52.00
Candida tropicalis	5	15.62	15.05	12.20–18.20
Acinetobacter	4	5.88	5.9	4.90–6.80
Acinetobacter species	4	40.8	41.3	32.60–48.00
Pseudomonas aeruginosa	4	16.82	16.96	6.48–26.88
Salmonella Typhi	4	15.25	16.56	10.80–17.08
Candida albicans	4	39.08	34.12	30.00–58.08
Serratia species	3	9.4	9.02	8.09–11.09
Salmonella spp.	2	11	11	4.08–17.91
Enterobacter sp.	2	29.9	29.9	13.01–46.80

Table-2. Organism wise Time to Positivity

Organism	Total number	Mean TTP (h)	Median TTP (h)	Range (h)
Candida parapsilosis	2	19.02	19.02	18.02–20.03
Candida non-albicans	2	29.92	29.92	3.84–56.00
Candida glabrata	2	50.5	50.5	49.00–52.00
Burkholderiacepacia complex	2	9.62	9.62	8.04–11.20
Aeromonas spp.	2	2.04	2.04	1.92–2.16
Proteus mirabilis	2	9.02	9.02	8.01–10.02
Candida krusei	1	56	56	56
Burkholderiapseudomallei	1	12.2	12.2	12.2
Klebsiella oxytoca	1	3.6	3.6	3.6
Proteus vulgaris	1	6.05	6.05	6.05
Pseudomonas species	1	22.8	22.8	22.8
Salmonella paratyphi A	1	15.84	15.84	15.84
Streptococcus pneumoniae	1	31.68	31.68	31.68

Table-2. Organism wise Time to Positivity

The large number of isolates was E. coli (n = 56), with a mean TTP of 12.10 hours and median of 6.21 hours showed in the table 2, among the Candida group TTP was generally prolonged. Candida spp. had a median of 52.00 hours while C.glabrata had a mean/median of 50.50 hours. The shortest mean TTP among organisms with ≥ 2 id isolates were observed for Aeromonas spp. (2.04 hours). Several organisms had very small sample size (n = 1–2); therefore, their mean/median TTP should be interpreted as descriptive value only rather than representative organism specific estimates.

The organism specific variation in TTP, with common Enterobacter and Staphylococci generally showing earlier positivity, whereas several non-fermenting gram-negative bacilli and Candida isolates demonstrated markedly prolonged positivity times. The difference between mean and median TTP for several organisms also indicates the presence of delayed-positive observations influencing the mean.

The Time-to Positivity (TTP) of the 259 isolated organisms was analysed across 35 organism groups using the Kruskal-Wallis H test, as shown in figure-3. The analysis demonstrated a statistically significant difference in TTP among the organism groups ($H = 116.606$, $df = 34$, $p < 0.001$).

When the major organism groups were compared, the difference in TTP was statistically significant (Kruskal-Wallis $H = 34.27$, $p < 0.001$).

Candida species demonstrated markedly longer TTP than the bacterial groups.

This finding is consistent with recent literature demonstrating significant pathogen dependent differences in blood culture TTP, including longer detection times for fungal organisms compared with many bacterial pathogens.

Procalcitonin and TTP

Blood culture-positive observations with documented Time-to-Positivity (TTP) and Procalcitonin (PCT) values were available for 240 of the 259 samples with TTP results, as shown in Table-3. The null hypothesis (H_0) started that there was no correlation between TTP and PCT, whereas the alternative Hypothesis (H_1) started that a correlation existed between TTP and PCT. Statistical significance was considered at $p < 0.05$. As multiple organism-wise correlations were assessed, the Benjamini-Hochberg procedure was applied to adjust for multiple comparisons, and the resulting adjusted p-values(q-value) were reported. The negative p=higher PCT associated with shorter TTP; positive p= higher PCT associated with longer TTP. Across all 240 isolates, there was a weak negative correlation between TTP and PCT Sperman's p= -0.270, $P < 0.00$, higher PCT values tended to be associated with shorter blood culture TTP.

Organism	n	Median TTP (h)	Median PCT	Spearman ρ	p-value	Interpretation
Escherichia coli	51	6.21	23.01	−0.377	0.0063	Significant negative
Klebsiella pneumoniae	33	5.01	29.1	0.048	0.79	No significant correlation
CoNS	28	9.64	3.37	−0.670	< 0.001	Significant negative
Acinetobacter Baumanni	15	8.02	3.05	0.03	0.917	No significant correlation
Enterococcus Faecalis	10	11.17	17.47	0.596	0.069	Positive, not significant
Burkholderiacepacia	8	11.52	28.52	0.252	0.548	No significant correlation
Enterococcus sp	8	13.34	12.32	0.488	0.22	No significant correlation
Non-fermenting GNB	8	35.28	0.96	0	1	No correlation
MRSA	7	6.07	4.43	0.306	0.504	No significant correlation
MR-CoNS	6	13.8	1.95	0.429	0.397	No significant correlation
Streptococcus sp	6	9.84	22.01	−0.032	0.952	No correlation
Candida spp	5	52	15.37	−0.918	0.028	Strong negative*
Candida tropicalis	5	15.05	41.55	−1.000	< 0.001	Strong negative*
Staphylococcus aureus	5	8.46	30.16	−1.000	< 0.001	Strong negative*
Acinetobacter	4	5.9	11.93	−0.800	0.2	Not significant
Acinetobacter species	4	41.31	0.7	0.4	0.6	No significant correlation
Candida albicans	4	34.13	13.5	−0.200	0.8	No significant correlation
Pseudomonas aeruginosa	4	16.96	16.72	−0.800	0.2	Not significant
Salmonella Typhi	4	16.56	0.97	−0.800	0.2	Not significant
Serratia species	3	9.02	15.37	−1.000	< 0.001*	Very small sample

Organism	n	Median TTP (h)	Median PCT	Spearman ρ	p-value	Interpretation
Other organisms,	22	—	—	—	—	Correlation cannot be calculated

Table-3. Procalcitonin and Organism wise Time to Positivity

The organism groups showing statistically significant negative correlations were; CoNS: p =-0.0670, p < 0.001 Escherichia coli: p = -0.377, p = 0.006, After adjustment for multiple comparisons using the Benjamini-Hochberg method, both remained statistically significant: CoNS : q < 0.001, E.coli : q = 0.025. The very strong correlations observed for S.aureus, Candida tropicalis and Serratia species should be reported cautiously because their sample size were only 5, 5 and 3 respectively.

Spearman rank correlation analysis was performed to evaluate the association between blood culture time to positivity(TTP) and Procalcitonin(PCT) levels among the culture positive samples. Overall, 240 blood culture isolates with corresponding TTP and PCT measurements were included in the analysis. A statistically significant weak inverse correlation was observed between TTP and PCT levels (Sperman's p = -0.270, p < 0.001), including that higher PCT concentrations tended to be associated with shorter blood culture TTP.

Organism wise analysis demonstrated a statistically significant inverse correlation between TTP and PCT among CoNS isolates (p=-0.670, p < 0.0001) and Escherichia coli isolates (P = -0.377, p = 0.006). this suggests that, within these organism groups, higher PCT levels were associated with earlier detection of microbial growth in the automated blood culture system.

For Klebsiella pneumoniae, no significant correlation was observed (p = 0.048, p = 0.790). similarly, no statistically significant association was demonstrated for Acinetobacter baumannii (p = 0.030, p = 0.917), Burkholderia cepacian (p = 0.252, p = 0.548), Enterococcus species, MRSA, MR-CoNS, Streptococcus species, and the other organism groups with adequate sample sizes.

Strong inverse correlations were observed in some organisms with small numbers of isolates, including Staphylococcus aureus, Candida tropicalis and Serratia species. However, these findings should be interpreted cautiously because of the limited sample size and should be considered exploratory rather than definitive.

The organism specific analysis therefore suggests that the relationship between PCT concentration and blood culture TTP is not uniform across all organisms. The significant inverse associations observed particularly among CoNS and E.coli indicate that elevated PCT may be associated with earlier blood culture positivity in selected organism groups.

Relationship between TTP & PCT

The scatter plot analysis demonstrates the relationship between time to Positivity (TTP) and procalcitonin (PCT) levels across the major bacterial isolates, as shown in Figure-4. E. coli showed a moderate inverse relationship, with higher PCT values generally observed at shorter TTPs, although considerable variability was present. Klebsiella pneumoniae demonstrated a moderate positive relationship, with PCT tending to increase with increasing TTP. In CoNS, the association between TTP and PCT was weak, with most observations clustered at relatively low PCT values despite variation in TTP.

For Acinetobacter baumannii, a moderate inverse pattern was observed, with higher PCT values predominantly occurring at shorter TTP. Enterococcus spp. showed a relatively strong positive relationship, with PCT tending to increase as TTP increased. S.aureus demonstrated an apparent inverse relationship between TTP and PCT; however, this finding should be interpreted cautiously because of the small number of observations.

Overall, the scatter plots indicate that the relationship between TTP and PCT varies considerably among different organisms, suggesting that the association is organism specific rather than uniform across all bloodstream isolates.

Organism-wise PCT analysis

Organism	n	Mean PCT	Median PCT	SD	Min–Max
Escherichia coli	51	25.45	—	—	0.02–89.12
Klebsiella pneumoniae	33	26.33	—	—	2.66–79.30
CoNS	28	4	—	—	0.04–31.52
Acinetobacter baumannii	15	4.46	—	—	0.06–19.90
Enterococcus faecalis	10	22.01	—	—	2.94–45.63
Enterococcus sp.	8	14.36	—	—	2.35–28.63
Burkholderiacepacia	8	30.32	—	—	20.47–45.84
Non-fermenting GNB	8	9.05	—	—	0.03–58.33
MRSA	7	4.23	—	—	0.23–8.01
MR-CoNS	6	14.55	—	—	0.11–65.62
Streptococcus sp.	6	28.21	—	—	0.53–86.22
Candida tropicalis	5	43.06	—	—	38.00–55.19
Candida spp.	5	27.99	—	—	3.71–100.00
Staphylococcus aureus	5	32.14	—	—	6.27–79.04
Acinetobacter	4	11.84	—	—	10.80–12.70
Acinetobacter species	4	0.71	—	—	0.48–0.96
Pseudomonas aeruginosa	4	17.74	—	—	2.26–35.28
Salmonella Typhi	4	4.58	—	—	0.35–16.02
Candida albicans	4	11.56	—	—	1.83–17.39
Serratia species	3	19.77	—	—	7.61–36.33
Proteus mirabilis	2	5.71	—	—	5.40–6.02
Enterobacter sp.	2	29.29	—	—	12.94–45.63
Candida parapsilosis	2	19.36	—	—	18.06–20.66
Candida non-albicans	2	15.96	—	—	3.43–28.48
Candida glabrata	2	0.46	—	—	0.43–0.49
Burkholderiacepacia complex	2	20.1	—	—	14.63–25.56
Aeromonas spp.	2	61.91	—	—	58.20–65.62
Burkholderiapseudomallei	1	13.04	—	—	13.04–13.04

Organism	n	Mean PCT	Median PCT	SD	Min–Max
Candida krusei	1	2.73	—	—	2.73–2.73
Proteus vulgaris	1	9.08	—	—	9.08–9.08
Klebsiella oxytoca	1	0.83	—	—	0.83–0.83
Salmonella spp.	1	0.33	—	—	0.33–0.33
Salmonella paratyphi A	1	13.18	—	—	13.18–13.18
Pseudomonas species	1	3.01	—	—	3.01–3.01
Streptococcus pneumoniae	1	2.03	—	—	2.03–2.03

Table-4. Organism wise Procalcitonin level analysis

The distribution of PCT levels was compared across the 35 organism categories using the Kruskal-wallis H test. The analysis demonstrated a statistically significant difference in PCT distributions among the organism groups ($H = 114.59$, $p < 0.001$), as shown in Table-4.

However, several organism groups have very small sample size($n = 1–2$), so their individual PCT estimates should be interpreted cautiously. The significant overall result indicates that PCT values are not distributed similarly across all organism categories, it does not by itself, establish which specific organism pairs differ.

Procalcitonin levels were evaluated according to the organism isolated from blood culture. The median and mean PCT concentrations were calculated for each organism group. As the organism variable was categorical and PCT was a continuous, non-normally distributed variable, comparison among organism groups was performed using the Kruskal-Wallis test. A statistically significant difference in PCT distribution was observed among the different organism groups ($H = 114.59$, $p < 0.001$). Higher mean PCT concentration was observed among patients with Aeromonas spp (61.91), Candida tropicalis (43.06), Staphylococcus aureus (32.14), Burkholderia cepacia(30.32) Enterobacter spp. (29.29), and Streptococcus spp. (28.21). In contrast, lower mean PCT concentrations were observed in CoNS (4.00), MRSA (4.23), Acinetobacter baumannii (4.46), and Salmonella Typhi (4.58). These findings indicate significant heterogeneity in PCT concentrations across organism; however, interpretation of organisms with small sample sizes should be cautious.

DISCUSSION

The present retrospective observational study provides a clinically relevant assessment of time to positivity (TTP) of blood culture processed by the automated Bact/Alert 3D system, with additional evaluation of organism-specific variation and its relationship with serum procalcitonin (PCT). In routine clinical microbiology practice, TTP is not merely an instrument-generated time interval, it can provide indirect information regarding the microbial burden present in the blood culture bottle and may assist

clinicians in interpreting the urgency and significance of a positive blood culture. In the present study, among 9,674 suspected patient blood culture specimens received during the 12month study period, 434(4.5%) yielded significant organism growth, of which 259 had documented TTP for analysis. The overall TTP had a mean of 14.36 ± 13.67 hours and a median of 9.21 hours, with an IQR of 6.00–16.28 hours. Importantly, 62.9% of the positive cultures became positive within 12 hours and 82.6% within 24 hours, demonstrating the capacity of continuous-monitoring automated blood culture systems to provide clinically actionable microbiological information relatively early after incubation. These findings are consistent with a large south Indian retrospective study of 38,606 positive blood cultures, in which 79% of cultures became positive within 24 hours and only 6% required more than 48 hours for detection [14]. The close agreement between these observations is particularly relevant because both studies represent real world laboratory practice rather than experimentally simulated bacteraemia.

The relatively early positivity observed in the present study is also consistent with the biological basis of automated blood culture detection. The Bact/Alert 3D system continuously monitors metabolic changes associated with microbial growth, principally through detection of carbon dioxide generated during growth. Consequently, TTP is influenced by the initial microbial inoculum, growth characteristic of the organism, blood volume, antimicrobial exposure before collection, specimen transport and loading time, and the culture medium and detection system used. Recent Indian work has emphasized that appropriate blood volume and other pre-analytical factors remain important determinants of culture performance and TTP. A 2025 Indian study using Bact/Alert 3D in paediatric blood cultures reported a median TTP of 18.13 hours and found that Gram negative organisms had the shortest median TTP at 11.39 hours. [15]. Thus, the relatively early positivity observed in the present study should be interpreted as the combined consequence of organism biology and laboratory workflow rather than as a fixed characteristic of the Bact/Alert 3D platform alone.

A major finding of the present study was the marked organism dependent variation in TTP. The organism wise distribution was right-skewed, with most isolates becoming positive relatively early but a smaller number showing considerably prolonged detection times. Escherichia coli was the predominant isolate (n = 56), with a mean TTP of 12.10 hours and median TTP of 6.21 hours. Klebsiella pneumoniae demonstrated an even shorter median TTP of 5.01 hours. In contrast, non-fermenting Gram-negative bacilli showed a markedly pronged median TTP pf 35.28 hours. Such differences are biologically plausible because TTP is strongly influenced by the growth kinetics and initial concentration of the organism. The Indian study involving 38,606 positive blood cultures similarly demonstrated that Gram negative pathogens generally became positive earlier than Gram-positive pathogens, with average TTPs of 12.5 and 15.4 hours, respectively [16]. The predominance of E.coli and K. pneumoniae in the present dataset is also comparable with contemporary Indian bloodstream infection studies, in which Enterobacterales remain prominent causes of culture positive bloodstream infection.

The statistically significant difference in TTP among the 35 organism categories in the present study (Kruskal-Wallis $H = 116.606$, $df = 34$, $p < 0.001$) further supports the observation that TTP is pathogen dependent. This finding should be interpreted as evidence of heterogeneity among organism groups

rather than as evidence that TTP alone can identify a particular species. The significant growth wise difference was also demonstrated when the isolates were considered according to the major categories, with Gram-positive, Candida and other organisms showing significantly different TTP distributions ($H = 34.27$, $p < 0.001$). The present data therefore reinforce the importance of considering organism category when TTP is incorporated into clinical interpretation. Recent literature likewise demonstrates that TTP differs according to pathogen type, with Gram-negative bloodstream infections generally showing earlier positivity than Gram-positive infections and fungal bloodstream infections tending to have longer detection times [17].

The relatively prolonged TTP observed among Candida isolates is an important observation from the present study. Candida spp. demonstrated a median TTP of 52 hours, while Candida glabrata had a median TTP of 50.5 hours and Candida albicans a median TTP of 34.12 hours. Although the numbers of individual Candida species in the present study were small, the overall tendency towards delayed positivity is consistent with Indian data. A prospective study from PGIMER, Chandigarh, published in the Indian Journal of Medical Microbiology, specifically evaluated TTP in fungaemia and demonstrated clinically useful variation among Candida species [18]. Similarly, a five-year study from a tertiary-care centre in Kerala involving 100 Candida-positive blood cultures processed on Bact/Alert 3D reported a median TTP of 34 hours for all Candida isolates' C. glabrata had a substantially longer median TTP of 70.5 hours, whereas C. tropicalis had a median TTP of 20.5 hours[19]. These observations support the present finding that fungal bloodstream infections should not be interpreted using the same TTP expectations as common bacterial bloodstream infections.

The organism-specific results also demonstrate why median TTP may be more informative than mean TTP for skewed microbiological data. For example, E. coli had a mean TTP of 12.10 hours but a median of only 6.21 hours, reflecting the influence of several delayed-positive cultures. Similar differences between mean and median were evident for MR-CoNS, non-fermenting Gram-negative bacilli, Enterococcus faecalis and Candida isolates. In a clinical microbiology dataset, delayed-positive observations may result from low initial organism burden, prior antimicrobial exposure, inadequate blood volume, intermittent bacteraemia or slower growth characteristics. Therefore, the median and IQR provide a more robust representation of the typical TTP distribution, while the mean remains useful for descriptive comparison.

The present study also identified an important relationship between TTP and serum PCT. Among 240 Culture-positive observations with paired PCT results, a statistically significant weak inverse correlation was demonstrated between TTP and PCT (Spearman's $p=-0.270$, $p < 0.001$). This indicates that higher PCT concentrations tended to occur in cultures that became positive earlier. Biologically, one possible explanation is that higher PCT levels may accompany a greater systemic inflammatory response and, in some cases, a greater circulating microbial burden, which could contribute to earlier microbial detection in blood culture. However, this relationship should be interpreted as an association rather than evidence of a direct causal relationship. PCT is a host-response biomarker and TTP is a microbiological growth-detection parameter; they represent different biological dimensions of bloodstream infection.

The organism-wise PCT analysis further demonstrated that the relationship between PCT and TTP was not uniform across all organisms. A significant inverse correlation was observed for E. coli (p=-0.377, p = 0.0063) and CoNS (p=-0.670, p < 0.001), and both remained statistically significant after Benjamini-Hochberg adjustment, with q = 0.025 for E. coli and q < 0.001 for CoNS. These findings suggest that, within these specific organism groups, increasing PCT concentrations were associated with earlier blood culture positivity. The E. coli finding is particularly biologically plausible because Gram-negative bloodstream infections can produce substantial systemic inflammatory responses, although PCT should not be considered a surrogate for bacterial load.

Recent Indian evidence supports the usefulness of PCT as an adjunctive rather than standalone marker in bloodstream infection. A 2026 south Indian study from JSS Medical College and Hospital evaluated PCT among culture positive bloodstream infections and found Gram-negative organisms predominated, with mean PCT levels of 37.87 ng/mL compared with 35.09 ng/mL among Gram-positive infections. The authors concluded that PCT may assist early BSI detection and preliminary differentiation of pathogen groups when interpreted together with clinical and microbiological findings [20]. Similarly, a 2025 prospective study from SRM Medical College Hospital and Research Centre evaluated PCT in culture-positive MICU patients, emphasising the potential role of PCT as an adjunctive biomarker in bacteraemia rather than a replacement for blood culture [21].

The absence of a significant TTP-PCT association in several organisms is equally important. K. pneumoniae, A. baumannii, Burkholderia cepacia, Enterococcus spp., MRSA, MR-CoNS and streptococcus spp. did not demonstrate statistically significant correlations in the present dataset. This heterogeneity indicates that the relationship between systemic inflammatory response and microbiological detection cannot be generalized across pathogens. Differences in organism virulence, tissue invasion, bacterial burden, host immune response, prior antimicrobial treatment and timing of PCT measurement may all contribute to this variability. Therefore, the overall inverse correlation should not be interpreted as a universal biological rule applicable to every bloodstream pathogen.

Strong inverse correlations were observed for S. aureus, C. tropicalis and Serratia spp., however, these observations require particular caution because only five, five and tree isolates, respectively were available for correlation analysis. A correlation coefficient of -1.000 in such small datasets may appear statistically striking but dose not provide sufficient evidence for a stable organism-specific biological relationship. The present study appropriately treats these observations as exploratory. This is an important methodological point because organism-wise correlation analysis can produce apparently strong coefficients when sample size is very small. Larger prospective datasets are required before such relationships can be considered reproducible.

The PCT distribution itself also varied significantly between organisms (Kruskal-Wallis H = 114.59, p < 0.001. Higher mean PCT concentrations were observed among Aeromonas spp., Candida tropicalis, S.aureus, Burkholderia cepacia, Enterobacter spp., and Streptococcus spp., whereas lower mean values were observed in CoNS, MRSA, A. baumannii and Salmonella typhi. The significant overall Kruskal-Wallis

result indicates heterogeneity among organism categories but dose not establish which individual organism pairs differ. Furthermore, several categories contained only one or two observations, and therefore their mean PCT values should not be interpreted as organism-specific reference values. This limitation is particularly important when interpreting apparently high values for Aeromonas spp. and apparently low values for some Candida or Acinetobacter categories.

The present findings also have implications for the interpretation of CoNS-positive blood cultures. CoNS showed a median TTP of 9.64 hours and a relatively strong inverse TTP-PCT correlation. However, CoNS represent an important interpretive challenge because they may represent either genuine bloodstream infection or contamination. TTP can contribute to this distinction, but it should never be used in isolation. Studies using automated Bact/Alert 3D systems have shown that true bacteraemia generally has shorter TTP than contamination, although substantial overlap remains [22]. Therefore, in routine practice, the interpretation of CoNS should incorporate the number of positive bottles or set, clinical features, presence of intravascular devices, repeat culture results, inflammatory markers and the overall clinical context. From a laboratory-practice perspective, the present findings reinforce the value of continuous monitoring and prompt communication of early positive blood cultures. More than four-fifths of the TTP documented cultures in this study became positive within 24 hours. A positive signal during this period should trigger rapid Gram staining, immediate communication of the preliminary result and expedited organism identification and antimicrobial susceptibility testing. TTP can therefore function as an additional laboratory parameter that complements, rather than replaces, Gram stain, organism identification and antimicrobial susceptibility testing. Recent Indian and international evidence continues to support the clinical importance of reducing the interval between blood culture positivity and organism-directed management [23].

An important strength of the present investigation is that it reflects routine tertiary care laboratory practice over a complete 12month period, rather than a restricted experimental population. The study includes a broad spectrum of bacterial and fungal isolates and combines microbiological TTP data with contemporaneous PCT measurements. The use of ono-parametric statistical methods, particularly Spearman correlation and Kruskal-Wallis testing, is appropriate given the skewed nature of TTP and the unequal organism group sizes. The use of Benjamini-Hochberg correction for multiple organism-wise correlations is an additional methodological strength because it reduces the likelihood of interpreting chance findings as statistically significant associations.

Several limitations should nevertheless be acknowledged. First, the study is retrospective and single centre, and therefore the findings may not be directly generalizable to other hospitals with different patient populations, blood culture practices or antimicrobial-use patterns. Second, PCT was available for only 240 of the 259 TTP observations, resulting in incomplete paired-data availability. Third, organism groups were highly unequal in size, and several organisms had only one or two isolates. Fourth, potential determinants of TTP such as exact blood volume, time from collection to loading, prior antibiotics exposure, number of positive bottles, severity of illness and source of infection were not incorporated into the correlation analysis. These factors are known to influence blood culture detection and may

confound the observed relationship between TTP and PCT. Fifth, because the study evaluates associations, it cannot establish that a higher PCT directly causes earlier culture positivity.

Overall, the findings demonstrate that TTP obtained from the Bcat/Alert3D system contains clinically meaningful organism-dependent information. Most significant bloodstream isolates were detected within the first 24 hours, while substantial differences were observed between bacterial and fungal organisms. The highly significant Kruskal-Wallis results demonstrate that TTP varies significantly according to organism category. Furthermore, the weak but significant inverse relationship between TTP and PCT in the overall cohort suggests that higher systemic inflammatory activity, as reflected by PCT, may accompany earlier microbiological detection in a proportion of bloodstream infections. However, the organism- specific findings clearly demonstrate that this relationship is heterogeneous and cannot be applied uniformly to all pathogens. The strongest reproducible associations in the present study were observed with E. coli and CoNS, whereas apparently very strong correlations in small organism groups should remain exploratory. In practical terms, TTP, PCT and organism identification should be regarded as complementary parameters, with their interpretation integrated with clinical findings, blood culture methodology and antimicrobial susceptibility results. This integrated approach is particularly relevant in tertiary care microbiology laboratories where rapid laboratory communication and appropriate interpretation of early blood culture signals are central to the management of suspected bloodstream infection.

CONCLUSION

The present study demonstrates that time to positivity (TTP) obtained from the automated Bact/Alert 3D blood culture system provides clinically relevant information regarding the detection of bloodstream infections and varies significantly according to the isolated organism. In this 12-month retrospective study, the majority of significant blood cultures became positive within the first 24 hours, emphasizing the importance of continuous automated blood culture monitoring and prompt communication od early positive results.

A significant difference in TTP was observed among the different organism groups, with Gram-negative bacteria generally showing earlier positivity, whereas several non-fermenting Gram-negative bacilli and Candida species demonstrated prolonged TTP. The significant Kruskal-Wallis findings further support the presence of substantial organism-dependent variation in blood culture detection time. These observations indicate that interpretation of TTP should take into consideration the organism isolated rather than applying a uniform TTP threshold to all bloodstream infections.

The study also demonstrated a weak but statistically significant inverse correlation between TTP and serum procalcitonin (PCT) in the overall culture-positive population, suggesting that higher PCT concentrations were associated with earlier blood culture positivity. However, this relationship was not consistent across all organisms. Significant inverse correlations were particularly observed among Escherichia coli and coagulase-negative staphylococci, while several other organisms showed no

statistically significant association. Strong correlations observed in organisms with very small sample sizes should be regarded as exploratory and require confirmation in larger studies.

Overall, the findings indicate that TTP and PCT provide complimentary information rather than interchangeable measures of bloodstream infection. TTP reflects the microbiological detection characteristics of the infecting organism, while PCT reflects the host inflammatory response. Their combined interpretation, together with clinical findings, Gram-stain results, organism identification and antimicrobial susceptibility testing, may improve the early laboratory assessment of patients with suspected bloodstream infection.

Declarations

Ethics approval and consent participate:

The study was approved by the Institutional Ethics Committee of SRM Medical College Hospital and Research Centre (Reference number- 8715/IEC/2023) and was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all participants prior to their inclusion in the study.

Consent for publication:

The authors have approved the final version of the manuscript for publication and consent to its publication in the journal.

Availability of data and materials:

Data will be made available on request.

Competing interests:

The authors declare that they have no competing interests related to this study.

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None.

Authors' contribution

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Figures

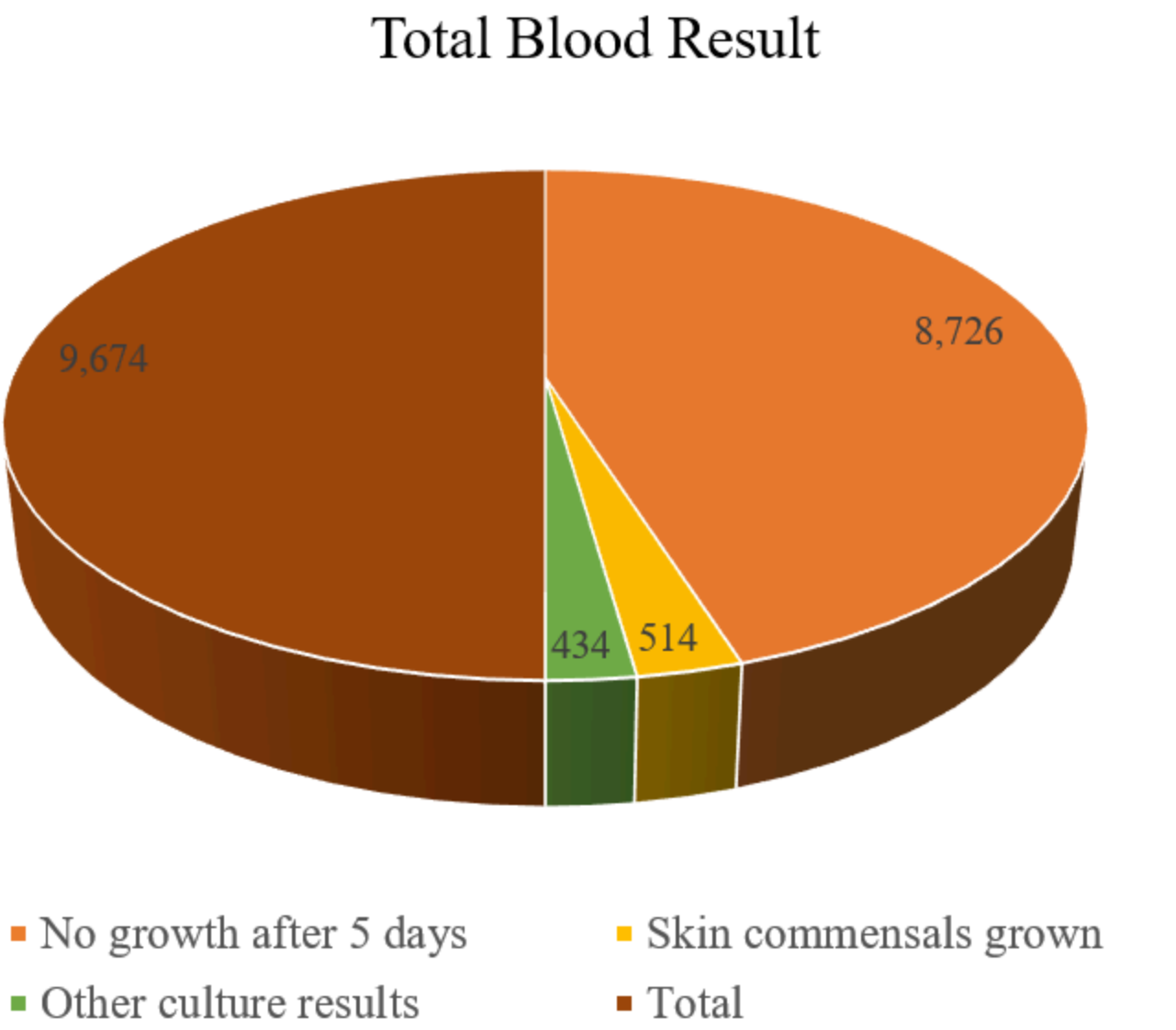


Figure 1

Total Blood Culture Result

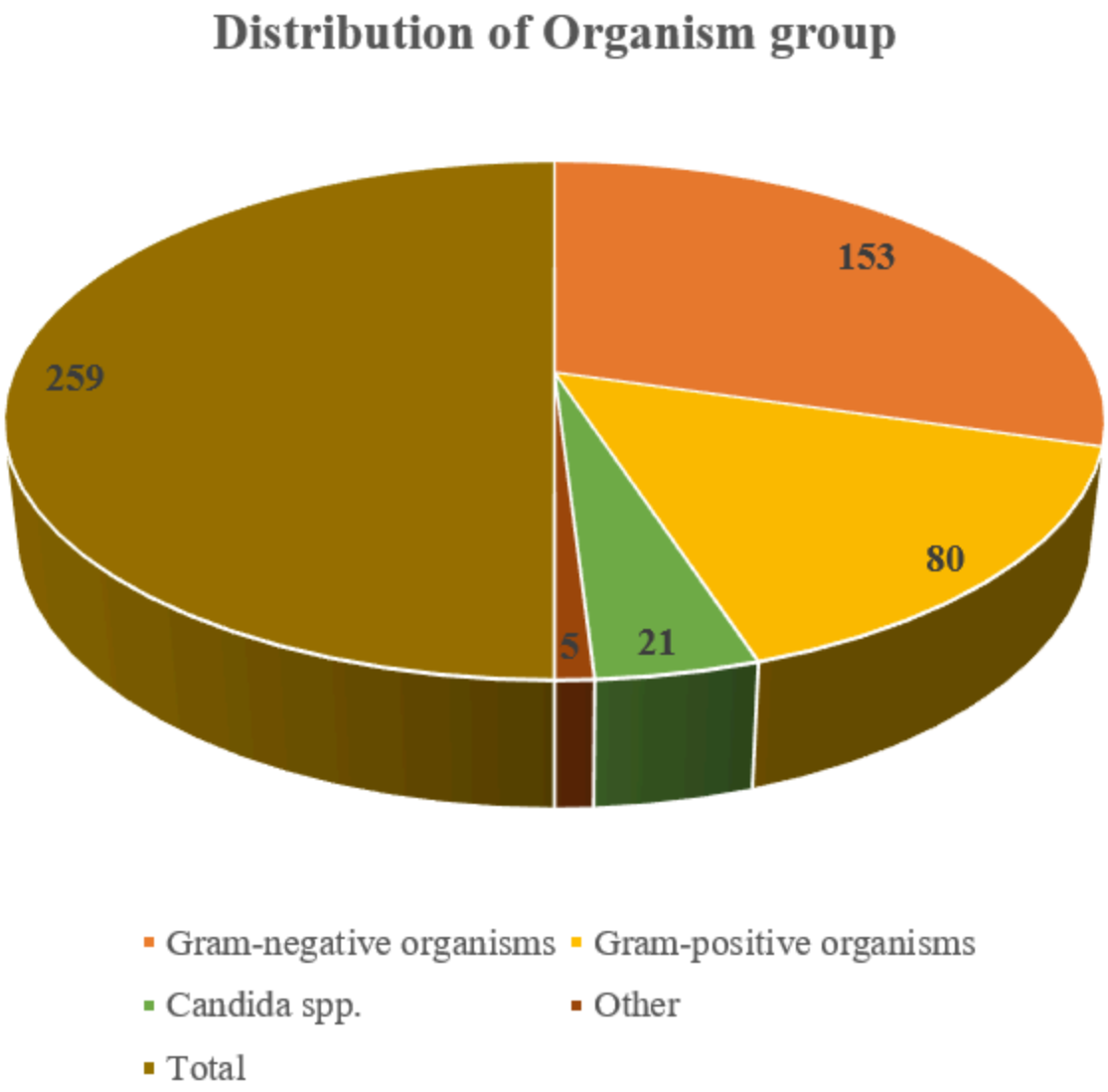


Figure 2

Organism group wise distribution

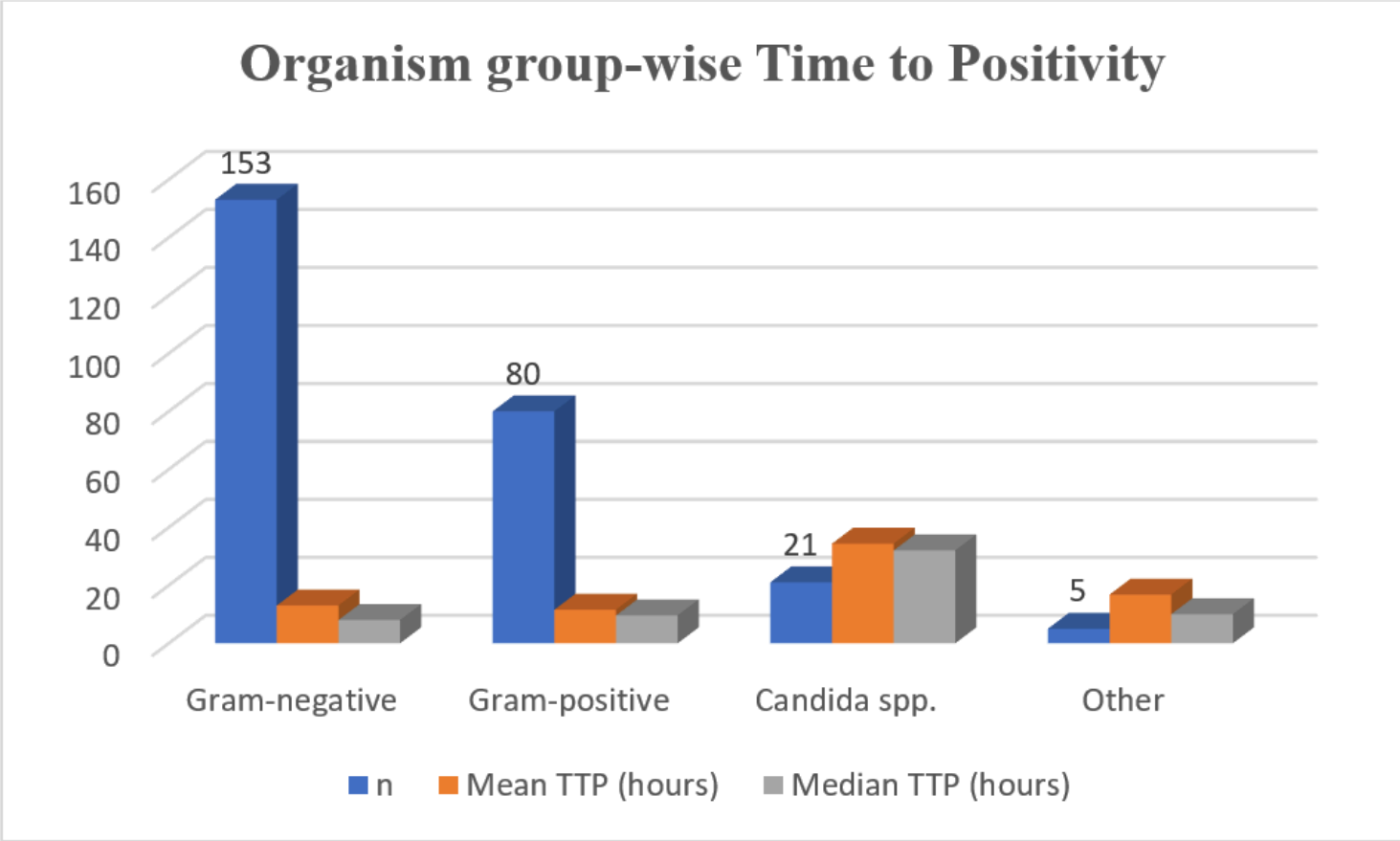


Figure 3

Organism group wise Time to Positivity

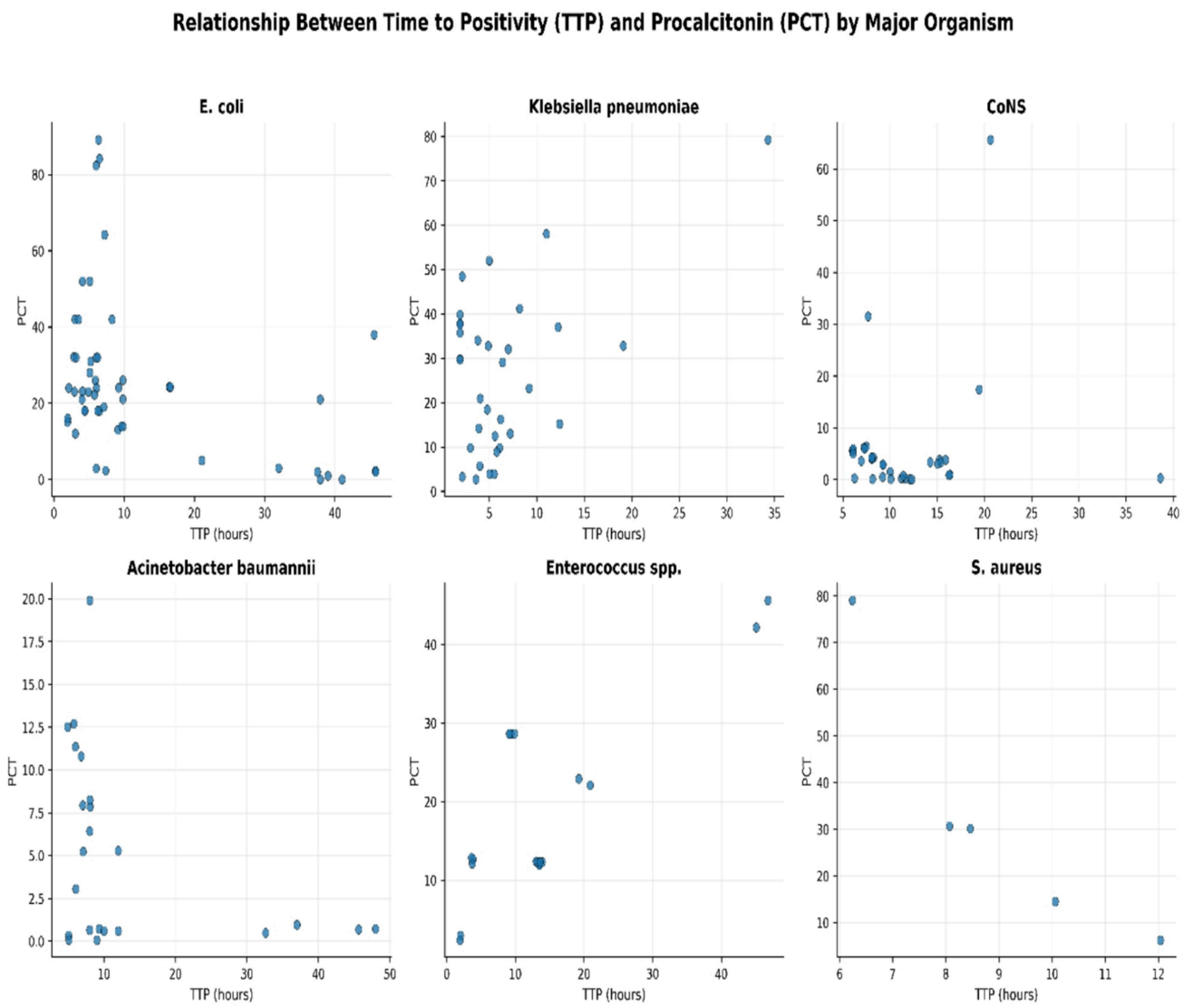


Figure 4

Relationship between Procalcitonin and Time to Positivity