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Early Detection of Sepsis: Comparative Analysis of Conventional vs Automatic Blood Culture and Few Inflammatory Markers

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Abstract:
BACKGROUND: Blood cultures are important tools in microbiology laboratories to detect and identify microorganisms in blood and their susceptibility to antibiotics Inflammatory markers also give early indication of sepsis. It also has important prognostic relevance by recommending appropriate antibiotic therapy and start the antibiotic therapy empirically when indication of sepsis we get from inflammatory markers. Automated blood culture systems are replacing traditional methods and it is important to compare them to understand their advantages and compare it with inflammatory markers for early prediction probability. The current study compares sepsis prediction by inflammatory markers and microbial detection rates and time to detection between a recently installed automated BacT/ALERT system and conventional approaches. Inflammatory markers also give early signs of sepsis. Conducted at a tertiary care facility in Kolkata, this prospective observational study included patients with suspected bacteremia and sepsis.
MATERIALS AND METHODS: Each of the 252 participants had two samples taken, which were then used to perform automated and traditional blood cultures as well as standardised tests for the inflammatory markers CRP, PCT, and MDA. Microorganisms' rate of detection and their time to detection were compared.
RESULTS: Overall, the automated, conventional, and inflammatory indicators indicated positive growths or sepsis prediction in 27.8%, 21.3%, and 33.44 of the samples, respectively. Inflammatory markers were found in 99.9% of the single isolates by the automated system, compared to 80% by the traditional method (p value 0.01). Within 24 hours, the automated system found 72.5% of the cases, and within 48 hours, it found 98.6% of the cases. 98% of cases were discovered by inflammatory markers in 24 hours and 99.9% in 48 hours. Comparatively, the conventional method only spotted 34% of the cases within 48 hours and none within 24 hours. For Gram positive bacteria, Gram negative bacteria, and fungus, the mean time to detection by the automated approach was 22.8, 18.24, and 23 hours, respectively.
CONCLUSION: In our situations, inflammatory indicators and automated blood culture systems outperform traditional blood culture techniques in terms of rate of detection and time to detection of microorganisms. Early findings disclosure makes it easier to start the right antibiotic therapy on time.
Keywords: Blood Culture, Inflammatory markers, Automated Blood Culture Systems, Bacteraemia, Sepsis.

I. BACKGROUND

Through a microbiological culture, blood culture can be used to identify the presence of living organisms in the blood. Blood is typically sterile, making blood cultures an essential tool for healthcare professionals. A positive blood culture result is very significant and signifies a life-threatening scenario that necessitates prompt attention. A positive blood culture can lead to a firm diagnosis, permit focused treatment against the relevant pathogen or organisms, and have prognostic significance. An essential role of clinical microbiology laboratories is the detection of bacteremia and fungemia, followed by antibiotic sensitivity testing, as this aids in the beginning of suitable and efficient antimicrobial therapy for a condition that could be fatal..

This necessitates the efficient application of all currently available techniques for the early detection of blood-infecting bacteria, including conventional techniques and automated blood culture systems. In most wealthy nations, traditional blood culture techniques have been replaced by automated and computerised techniques, whereas emerging nations like India are still in the process of upgrading. The availability of various systems, each claiming to be superior to others in various ways, is the outcome of technological advancements.[1,2,3,4]

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Automated systems which perform well in developed countries may not achieve the same results in the Indian context. In this study an automated blood culture system that has been installed in a tertiary care centre in Kerala is being compared with the conventional blood culture system to understand the additional benefits obtained if any with regard to rate and time of detection of micro-organisms thus resulting in better clinical outcome.

II. MATERIALS AND METHODS

A. Study Design and Setting

The study has a prospective observational design. Sample size was calculated as 240.Patients admitted with fever and or sepsis in Medicine, Paediatrics and Infectious Diseases department was included. Extremely low birth weight infants and hemodynamically unstable patients were excluded from the study.

B. Study Procedure

The study commenced after acquiring written permission from the Institutional Ethics Committee. Blood samples were collected after obtaining informed consent. Blood was collected under strict aseptic precautions. After locating a suitable vein the site was disinfected with 70% ethanol, then 1% povidone iodine and again ethanol. Blood was then withdrawn using sterile needle and syringe. The syringe was replaced with fresh sterile needle and then inoculated into the bottle. Two separate samples were taken from the same patient within 1-3 hours interval. Most of the samples were collected before giving antibiotics. Inflammatory markers like CRP, Procalcitonin quantitative and MDA was run from Clot vial 5 ml blood collected during first time collection of Blood Culture.

One sample was inoculated into conventional blood culture bottle in broth with 1:10 dilution and other into automated blood culture bottle. Second sample was inoculated into automated blood culture bottle (AutoBCS) Bact/ALERT system.

The medium used for conventional blood culture system is Brain Heart infusion broth with 0.05 % Polyanithol sulfonate. Conventional blood culture bottles were incubated at 37^0 C for six hours. Subculture was done after six hours and then on daily basis to Blood agar and MacConkey agar for 10 days. Gram staining was also done at the time of subculture. Direct antibiotic sensitivity was done based on Gram reaction. If there was no growth obtained after regular repeated subculture for 10 days, it was reported as 'Sterile after 10 days of incubation'. If the growth was obtained, colonies were identified by Gram staining along with standard biochemical tests and appropriate antibiograms on Mueller Hinton agar for each isolate.

If any growth was detected on Automated Blood Culture Systems (AutoBCS), a direct Gram staining of blood culture media was performed along with direct biochemical tests and direct antibiotic sensitivity tests for early reporting. Subculture was done on Blood agar and MacConkey agar, colonies identified and antibiotic sensitivity was done for each isolate. If no growth detected it was reported as 'Sterile after 10 days of incubation'. In addition a terminal subculture was done from the AutoBCS bottles to detect any false negatives. The results of the blood culture were entered in EpiData version 3.1 and analysed using EpiInfo software version 3.5.2. Analysis included a comparison of detection rates and time taken for detection between conventional and automated methods of blood culture.

III. RESULTS

There were a total of 252 participants in the study from whom two samples of blood was collected for inoculation into conventional and AutoBCS Bottles.

The subjects ranged from age of one day to 81 years. Male subjects accounted for 58.9% and females 41.1%. Neonates and infants less than one year constitute one-fifth of the study sample.

55.7 percent of the study subjects were admitted in the General Medicine ward or Intensive Care Units, while 36.7 percent was admitted in the Paediatric department. Patients admitted in the Infectious Diseases department constituted the rest. Almost half the subjects had a provisional diagnosis of sepsis while 37.5 percent fever. Urinary tract infections, Pneumonia and Meningitis constituted rest of the study subjects.

Among 252 sample pairs 75 were predicted by inflammatory markers , 71 (28.6 %) had positive blood culture results either by conventional, automated or both blood culture methods. Single isolates were found in 65 (26.2 %) sample pairs. Among the six samples which gave a mixed growth five were detected by the AutoBCS while one sample gave a mixed growth in both methods.

Among the 71 samples with positive growth, the automated system detected 69 (97.2%) while conventional detected 53 (74.7%) (Refer Table No. 1). Among the positive samples 18 (25.4 %) were detected only by the automated system while 2 (2.8%) were detected only by the conventional method. (Refer Table No. 2).

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Mcnemar's Chi Square test was done to compare the rate of detection between the two methods. The AutoBCS system had a significantly greater rate of detection than the conventional methods. (P value <0.001)(Refer Table No. 3).

Among the 65 sample pairs with single isolates the automated system detected 63 (96.9%) while the conventional system detected only 52 (80%). The automated system had a significantly greater rate of detection than the conventional method even after removing the mixed growth samples from analysis (Mcnemar's Chi Square P value <0.01).

Inflammatory markers were found to be best in predicting early indication of sepsis.

Early detection of pathogens facilitates initiation of appropriate antibiotic therapy and thus has prognostic significance. On comparison between conventional and automated methods, it was found that the automated system detected 12(17.4 %) cases within 12 hours and 50 cases within 24 hours of incubation, while none was detected by the conventional method within 24 hours. (Refer Table No. 4).

The mean time of detection of all microorganisms by automated system was 20.88 hours. The Gram negative bacteria had a lower mean time of detection of 18.24 hours compared to 22.8 hours for Gram positive bacteria but the difference was not statistically significant. Candida which was the sole fungal species isolated had a mean time of detection of 23 hours. (Refer Table No. 5)

Among the organisms isolated by both methods 53.5 percent were Gram negative organisms and the rest were Gram positive organisms.

Klebsiella pneumoniae, Coagulase Negative Staphylococci and Pseudomonas aeruginosa were the three most common organisms isolated accounting for 13 (18.3%) , 13 (18.3%) and 11 (15.5%) cases respectively. Candida growth was obtained in 5 (7%) cases while mixed growth was obtained in 6 (8.5%) cases. All the five Candida growth were detected by both the AutoBCS and conventional systems.

C. Antibiotic Sensitivity of Gram Positive Bacteria

Among the total of four isolates of Staphylococcus aureus, two were resistant to Cefoxitin (MRSA). But these isolates were sensitive to Vancomycin, Linezolid, Clindamycin and Rifampicin. Methicillin Sensitive Staphylococcus aureus (MSSA) were sensitive to Gentamicin, first generation Cephalosporin, Erythromycin and Amikacin. All of them were resistant to Penicillin. All MSSA isolates showed sensitivity to Vancomycin, Linezolid, Clindamycin and Rifampicin.

All isolates of Staphylococcus epidermidis were sensitive to Amikacin, Vancomycin, Clindamycin and Rifampicin while 12 isolates were Cefoxitin resistant.

Of the three Group D Streptococci isolates two were Enterococcus fecalis and one was an Enterococci other than bovis. Enterococcus fecalis were resistant to Penicillin and were sensitive to Ampicillin. Enterococcus other than bovis was sensitive to both Penicillin and Ampicillin.

All the three Group D Streptococci were sensitive to Vancomycin.

Streptococcus pneumoniae was sensitive to Penicillin, Erythromycin, Cephalosporin and Vancomycin.

D. Antibiotic Sensitivity of Gram Negative Bacteria

All the isolates of Enterobacteriaceae showed 100% sensitivity to Imipenem.

Klebsiella pneumoniae isolates showed maximum sensitivity to Imipenem, Cefoperazone + Sulbactam, Piperacillin-Tazobactam and then to Amikacin.

E. coli isolates also showed similar sensitivity pattern with maximum sensitivity to Imipenem, Amikacin, Cefoperazone-Sulbactam, Piperacillin- Tazobactam and Ciprofloxacin.

The isolates of Pseudomonas aeruginosa were sensitive to Ceftazidime (III generation Cephalosporin), Piperacillin-Tazobactam, and Imipenem. 90.9 percent of Pseudomonas aeruginosa isolates were sensitive to Amikacin and Ciprofloxacin. Only 63.6 % showed sensitivity to Gentamicin.

All the Acinetobacter baumannii isolates were sensitive to Imipenem and Cefoperazone-Sulbactam (100%). Two isolates were sensitive to Ciprofloxacin. Only 33.33% of isolates were Amikacin and Piperacillin-Tazobactam sensitive.

Acinetobacter baumannii isolates were 100% resistant to Ampicillin, I and III generation Cephalosporins.

All the Salmonella typhi and paratyphi isolates were sensitive to Ceftriaxone, Ciprofloxacin and Co-trimoxazole. But among the Paratyphi A only 66.6% were sensitive to Chloramphenicol.

Minimum inhibitory concentration of Ciprofloxacin was done along with disc diffusion test.

Flavobacterium meningosepticum isolated was sensitive to Vancomycin, Imipenem, Cefoperazone-Sulbactum and Ciprofloxacin.

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IV. DISCUSSION

The present study is a single centre comparative study for comparing the performance of the inflammatory markers, automated blood culture system with the conventional blood culture system. The study took place in the settings of a tertiary care centre which also acts as a referral centre, where timely diagnosis and early initiation of therapy has a prognostic significance. The Microbiology laboratories play an important role in rapid detection and identification of bacteraemia and initiation of appropriate antibiotic therapy. The performance of the automated blood culture system with respect to rate of detection and time to yield was compared to the conventional method. The study provides an opportunity for continuous quality improvement and compliance evaluation to ensure that the potential benefits of newer blood culture technology are optimally utilised.

Positive blood cultures were obtained in 71 (28.6%) sample pairs while single isolates were obtained in 65 (26.2%) sample pairs in this study. In a study involving suspected cases of neonatal sepsis alone the rate of positivity was 39 percent with automated system and 14 percent with conventional system.[5]

The rate of positivity varies from hospital to hospital and the rate of positive cultures would be increased when blood is taken from moribund patients.[3] The overall positivity also depends on the rate of contamination. The higher than average rate of blood culture positivity obtained can be attributed to the fact the study was conducted in patients with a clinical diagnosis of sepsis or suspected sepsis from the departments of General medicine, Infectious Diseases and Paediatrics. Patients from both the wards and intensive care units were also included in this study.

The automated system detected 97.2 percent of the positive samples while the conventional method detected only 74.7 percent of the positive samples. The automated system had a significantly greater rate of detection than the conventional method even after removing the mixed growth samples from the analysis which are assumed to be due to contamination. The better performance of automated system in isolation of microbes have been compared with conventional as well as manual continuous systems and found to be superior.[6] In a comparative study of the paediatric version of automated blood culture system and the conventional method, the automated detected 90 percent of clinically significant isolates compared to 71 percent by the conventional method.[7] In the present study the AutoBCS detected 96.9 percent of the single isolates while the conventional method detected 80 percent of the single isolates. These findings show that the AutoBCS system has a good isolation performance and is a reliable and better alternative to the conventional system in our settings.

In the present study the automated system detected 17.4 percent of the positive cases within 12 hours of incubation and 72.5 percent of the cases within 24 hours of incubation. All the positive cases except one sample with a mixed growth were identified within 48 hours of incubation. This is consistent with earlier observations that incubation periods of five days are sufficient for automated blood culture systems and organisms identified beyond five days are usually contaminants.[8,9] In comparison, the conventional method of blood culture detected none of the cases in the first 24 hours. The conventional method detected 34 percent, 75.5 percent and 88.7 percent of the cases within 48, 72 and 96 hours of incubation respectively.

Similar findings are also reported in a study wherein the AutoBCS performed better by detecting 30 percent of cases within 12 hours and 100 percent of cases within 48 hours. [5]

The mean time to detection of all positive culture was 20.88 hours in the present study. Gram Positive organisms had a slightly longer time to detection when compared to Gram negative organisms. The mean time to detect Candida growth in the present study was 23 hours which is comparable to other studies.

Horvath et al reported a mean time to growth detection of 25.6 hours for Candida for BacT/ALERT when compared to 27.3 hours for Bactec system.[10]

In the present study as soon as growth was detected by the automated system a Gram staining was done along with the sub-culture. Gram staining itself could guide empirical therapy to a certain extent.[11] If the Gram staining revealed Gram negative organisms, direct biochemical reactions were also attempted to identify the organisms early to facilitate early reporting.

In the conventional blood culture method biochemical reactions are usually performed after detecting growth in subcultures. Direct biochemical reactions with the incubated AutoBCS media helped in the identification of organisms and they were confirmed by subsequent tests using the sub-culture growths. Thus the detection and identification of pathogens especially Gram negative bacteria could be advanced by as much as 24 hours. This helps the clinicians to initiate appropriate antibiotics as early as possible.

In addition the alarm system in the AutoBCS facilitates early sub-culture of the specimen, thus facilitating early antibiotic sensitivity testing and reporting. This is in contrast to the conventional system where the initial subculture is done after six hours and then repeated every 24 hours. This is especially useful in facilities with less number of staff to monitor the daily load of blood cultures. The work load is considerably reduced as the laboratory staff does not have to do subcultures in all the cases.[3]

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Hardy et al found a 0.2 percent positivity among terminal subculture of negative AutoBCS bottles.[8] Another study from Korea which evaluated the negative AutoBCS results using terminal sub-cultures found 2.6 percent of the sub-cultures to be positive.[12] Automated systems are also good for culturing other sterile fluids.[13]

In the present study 53.5 percent isolates were Gram negative organisms and 46.5 percent were Gram positive organisms. Klebsiella pneumoniae, Coagulase Negative Staphylococci and Pseudomonas aeruginosa were the three most common organisms isolated accounting for 13(18.3%), 13(18.3%) and 11 (15.5%) cases respectively. Candida growth was obtained in 5 (7%) cases while mixed growth was obtained in 6 (8.5%) cases. All the five Candida growth were detected by both the AutoBCS and conventional systems.

Although automated continuous-monitoring blood culture systems are both rapid and sensitive, false-positive and false-negative results still occur. In the present study the conventional method detected two cases of Coagulase negative staphylococci that were not detected by the AutoBCS. They were most likely to be contaminants as they were also not detected by terminal sub-cultures from the AutoBCS bottles.[14]

A sentinel surveillance study showed that infections due to Gram-negative organisms are increasing and E.coli was the most common organism isolated.[15,16] Present study showed Klebsiella pneumonia followed by Pseudomonas aeruginosa as commonest Gram negative bacilli isolated. Nosocomial infections with multidrug resistant strains are increasing nowadays.[17,18,19,20]

Even though the study was focussing on bacterial pathogens, Candia isolates were also obtained and were the fourth most common cause of blood infections. Three of the patients were given antifungal treatment.[21]

V. CONCLUSION

The present study was a comparative study on conventional and automated blood culture system with respect to rate and time of detection of blood culture. About 78 percentage positive samples were detected by Auto BCS in first twenty four hours which enabled early processing and reporting. Rate of detection of the Auto BCS was also significant when compared to conventional. Specimen handling could be reduced by Auto BCS. The mean time to detection by the automated method was 22.8, 18.24 and 23 hours for Gram positive bacteria, Gram negative bacteria and fungi respectively. They can be a valuable tool for the early detection and identification of blood pathogens. This improves the prognosis of those patients admitted with fever and or sepsis. The rapid and reliable detection of blood stream infections helps in timely initiation of appropriate antibiotics. Automated blood culture systems are a reliable alternative to conventional blood culture systems.

	Conventional Method	Automated	Inflammatory markers cumulative (CRP,PCT & MDA)
Culture Positive	53	69	Early prediction 75
Sterile	199	183	123
Total	252	252	252
Table 1. Overall rate of detection in Automated and Conventional Culture methods			

Both Positive	51
Conventional Alone	2
Automated alone	18
Both Sterile	177
Total	248
Table 2. Culture positivity of all sample pairs	

	Conventional Method	
Automated Method	Positive	Sterile
Positive	51	18
Sterile	2	177
Table 3. McNemar's Chi Square test for significance (P value <0.001)		

	Conventional Method			Automated Method		
Day	Frequency	Percentage	Cumulative Percentage	Frequency	Percentage	Cumulative Percentage
0	0	0	0	50	72.5	72.5
1	18	34	34	18	26.1	98.6
2	22	41.5	75.5	1	1.4	100
3	7	13.2	88.7			
4	5	9.4	98.1			
5	1	1.9	100			
Total	53	100		69	100	

Table 4. Comparison of Conventional and Automated methods by day of detection

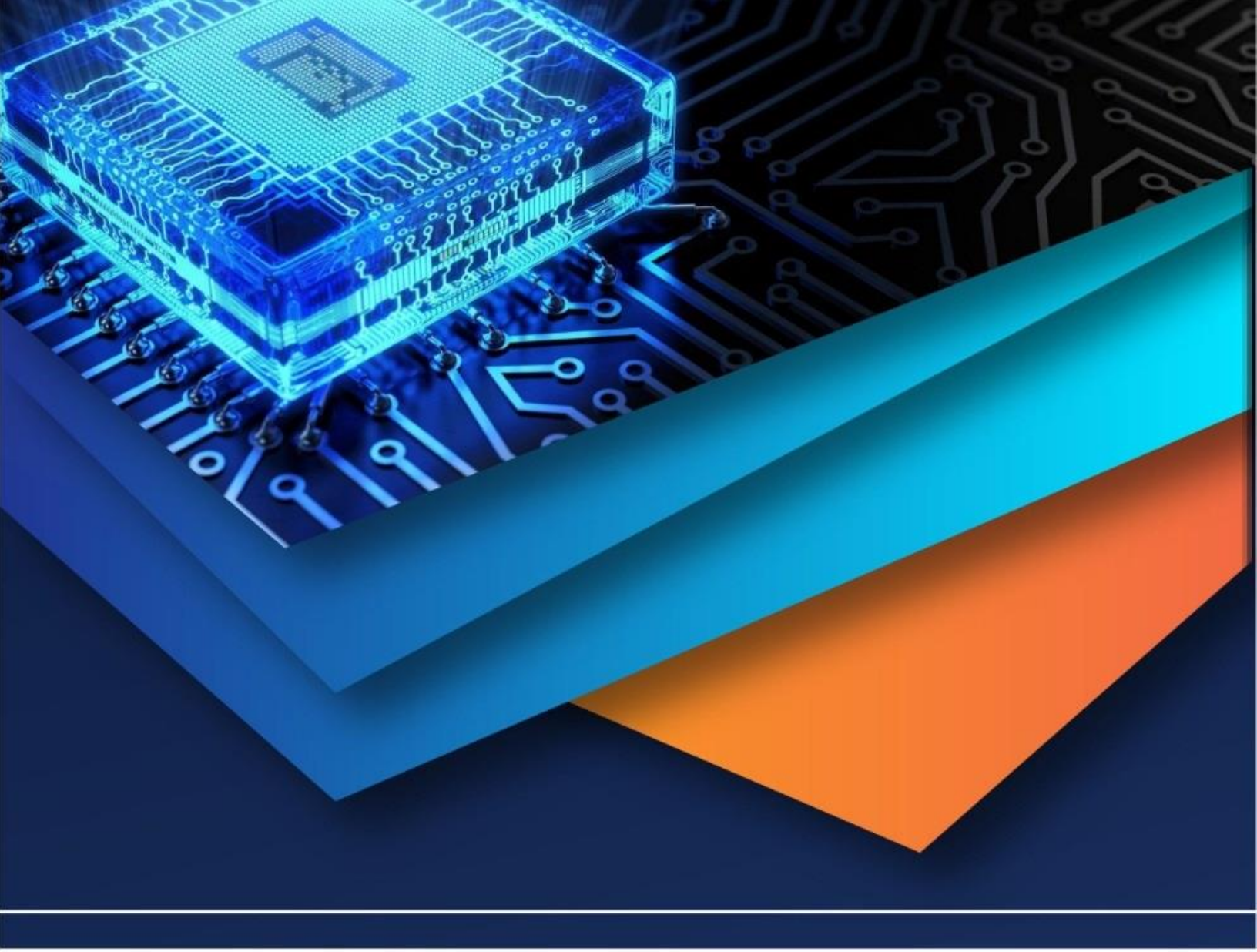
	N	Mean	Standard Deviation	
Gram Positive	31	0.9955 Days (23.89 Hours)	0.57	P- value 0.058
Gram Negative	38	0.7674 Days (18.42 Hours)	0.41	
Total	69	0.8699 Days (20.88 Hours)	0.5	

Table 5. Mean time of detection in Automated method

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