Bacterial Blood Isolates in Children: Conventional vs. Bactec Automated Blood Culture System in a Tertiary Health Centre in Gombe, North East Nigeria

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Abstract

Background/Aim: Blood culture is critical in the diagnosis and treatment of blood stream infections (BSIs) especially in children. BSIs are among the most common cause of morbidity/mortality and blood culture has remained the gold standard for diagnosis. We sought to compare Blood Culture Isolates (BCI) from conventional and Bactec automated blood culture system (ABCS) among paediatric patients at the Federal Teaching Hospital Gombe (FTHG) Nigeria. Methods: BCI in children (0 - 18 years) by conventional method from 2008-2012 and Bactec Automated culture system from 2015-2020 were retrieved from the clinical microbiology laboratory register. Information analyzed included, age, sex, month, and year and blood culture isolates. Results: There were 5276 (56.9% males, 43.1% females) and 1169 (54% males, 46% females) Blood Culture Isolates by CM and ABCS respectively. Overall positive culture isolates were 9.7% (515/5276) in CM and 45.9% (536/1169) in ABCS (p = 0.01). Positivity rate in newborn was 13.3% (282/2114) by CM and 40.9% (219/263) by ABCS (p = 0.01); under-5 was 10.5% (448/4253) vs. 37% (359/873) (p = 0.01); Gram positive 32.6% (172) vs. 65% (759) (p = 0.01; Gram negative 55% (2910) vs. 34% (397) (p = 0.01). Staph aureus 22% (114/515) by CM vs. 61.9% (332/536) by ABCS (p = 0.01); Klebsiella 24.9% (128/515) by CM vs. 7.5% (40/536) p = 0.01 in ABCS, E. coli 8.9% (46/515) vs. 2.1% (11/536) p = 0.01; Proteus vs. 1.1% (6/515) by ABCS, Pseudomonas 3.3%
(17/515) vs. 5.6% (30/536) $p = 0.05$, *Alkaligenes* 1% (5/515) vs. 8.2% (44/536) $p = 0.01$ and *Citrobacter* 1% (5/515) vs. 8.4% (45/536) $p = 0.01$. **Conclusion:** Blood culture yield was five times higher with Bactec compared with Conventional method.

**Keywords**

Children, Blood Culture, Isolates, Manual, Automated

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1. **Introduction**

Globally, bloodstream infection affects about 30 million people with 3 million newborns and 1.2 million children suffering from sepsis annually [1]. The rate of BSI is estimated to be 3 - 20 times higher in developing countries due to lack of adequate and regular microbiology laboratory in these countries [2]. While sepsis and bloodstream infections are not interchangeable bloodstream infections cause 25% - 30% of sepsis cases [3].

In 2017, almost half (20 million) of all estimated sepsis cases worldwide occurred in children under 5 years of age and in 2018, an estimated 15% of all neonatal deaths globally were due to sepsis [4]. Significant regional disparities in incidence and mortality exist with the highest rates in lower-middle-income countries especially sub-Saharan Africa and South East Asia [5].

Reliable and accurate diagnosis of these infections is therefore of utmost importance. Blood cultures are the reference method for diagnosis of BSI [6]. World Health Organization (WHO) defines blood cultures (BC) as a priority specimen for Antimicrobial Resistance (AMR) surveillance and it is recommended to prioritize key clinical specimens in resource-limited settings [7]. In Nigeria, a situation analysis of antimicrobial use [8] and a National Action Plan for AMR [9] prioritized the use of blood culture in diagnosing BSIs. Conventional/“Manual” blood culture systems rely on the use of appropriate blood culture bottles, which are placed in a conventional static incubator and are inspected daily to visually detect signs/evidences of growth of micro-organisms. In the automated system, the carbon dioxide production by micro-organisms in the bottles is continuously monitored, either by colorimetric or fluorescent detection. During incubation, the bottles are continuously agitated in the automated equipment [6].

Automated blood culture systems for incubation and growth monitoring have become the standard in high-income countries (HICs), but are still relatively expensive and not universally available for implementation in most LMICs [10]. Earlier Reports [11] [12] [13] and studies [14] [15] [16] from developing countries showed that automated systems show better performance than manual systems in terms of yield, sensitivity and especially speed of growth and overall turnaround time.

In a systematic review [17] of newborn blood cultures in Nigeria, the use of
manual culture method accounted for 89% of techniques used with few reports [18] [19] [20] [21], of automated systems. The BACTEC blood culture system yield was of 41% [22].

To the best of our knowledge, there are very scarce reports in Nigeria comparing the manual and automated blood culture systems in diagnosing BSIs. We therefore aimed to compare these two systems in terms of type and yield of blood culture isolates in children in our health facility.

2. Methodology

2.1. Study Location

The Federal Teaching hospital Gombe (FTHG) is currently a 500 bedded health facility which started providing health service to the public in the year 2000 with full-fledged Paediatric Department accredited for postgraduate residency training in Paediatrics in 2010. The Medical Microbiology Department of FTHG transitioned fully to automated blood culture system in 2015 from the conventional/manual system which was used at inception. Polymerase Chain Reaction (PCR) capacity exists in the department particularly for some viral infections such as HIV and Hepatitis.

2.2. Subjects

Blood culture samples from consecutive children admissions between 2008-2012 and 2015-2020 with suspected blood stream infections or sepsis were obtained using the Hospital standard procedure which was communicated regularly to the departmental staff by Paediatric infectious disease unit and the Medical Microbiology Department.

BCI in children (0 - 18 years) by conventional method from 2008-2012 and Bactec Automated culture system from 2015-2020 were retrieved. Information analyzed included, age, sex, month, and year and culture growth/identity of microorganisms. While the cost of a conventional test was N1000 or $2.5, that of Bactec was $12.5 or N5000 (At an official exchange of Naira 400/US$)

The BD Bactec(R) 9050 instrument which is designed for the rapid detection of microorganisms in clinical cultures of blood was used.

2.3. Principle

The Blood sample to be tested is inoculated into the vial which was entered into the Bactec 9050 for incubation and periodic reading. Each vial contains a sensor which detects increases in CO2 produced by the growth of microorganisms. The sensor was monitored by the instrument every ten minutes for an increase in its fluorescence, which was proportional to the amount of CO2 present. A positive reading indicates the presumptive presence of viable microorganisms in the vial which are subsequently sub cultured for identification and antibiotic susceptibility testing. Clinical and Laboratory Standards Institute (CLSI) guideline for antibiotic susceptibility testing was used.
2.4. Quality

Assurance was ensured and maintained in accordance with our hospital laboratory standard protocol for quality control and assurance.

2.5. Data Analysis

Data were entered into the EPIinfo version 3.5.1 software and analyzed. Automated and manual BC were compared in terms of proportion positive and recovery of different bacteria. Statistical difference between automated and manual BC were calculated using chi and Fischer’s exact test. A p-value below 0.05 was considered as statistically significant.

2.6. Ethical Approval

Approval for this study was received from the Ethical Research Committee of the Federal Teaching Hospital Gombe.

3. Results

Table 1 shows there were 5244 and 1169 blood cultures by Conventional and Bactec automated methods respectively in the two reporting periods. Newborns constituted 40% and 31% of the blood cultures by CM and ABCS respectively. Blood culture was Positive in 45.9% by Bactec and 9.8% by CM method and the difference was statistically significant (p = 0.001). There was no significant difference between gram positive and gram-negative bacterial culture isolates in the two blood culture categories. (p = 0.848), within each culture method, gram negative pathogens predominated. (p = 0.001) More males than females had positive blood cultures in both test methods, however this was also not significant statistically. (p = 0.303). In all childhood age categories, the Bactec Automated culture method had statistically significant positive blood culture yield when compared with the conventional method (p = 0.00).

In Table 2, while the five dominant bacterial isolates in order of frequency by CM are Klebsiella species, Staphylococcus aureus, Escherichia coli, Enterococcus, and Proteus species, on the other hand Staphylococcus aureus, Alcaligenes, Citrobacter, Klebsiella and Pseudomonas were the leading pathogens by the Bactec Automated system. The distribution was statistically significant for Klebsiella p = 0.001, Staphylococcus aureus p = 0.001, Alcaligenes p = 0.001, Citrobacter p = 0.001, Escherichia coli p = 0.001, Providencia p = 0.001 and salmonella p = 0.001. The difference in the distribution of other blood culture isolates between the two culture methods is not statistically significant. Potential contaminants were reported in CM and none in ABCS (P = 0.001).

In Table 3 Klebsiella was the leading isolate in the newborn by CM but by ABCS, Staphylococcus aureus was the dominant pathogen. In order of decreasing frequency, Staphylococcus aureus, Escherichia coli, Enterococcus and Enterobacter were isolated by CM in the newborn; in the ABCS and by decreasing frequency, Alcaligenes, Citrobacter, klebsiella and pseudomonas were the most
Table 1. Age, Sex and blood culture in children 0 - 18 years.

|                         | Conventional Blood culture methods 2008-2013 | Bactec Automated blood culture system 2015-2020 | X²   | p-value |
|-------------------------|---------------------------------------------|-----------------------------------------------|------|---------|
| Sex                     |                                             |                                               |      |         |
| Male                    | 2984 (57.0)                                 | 661 (56.5)                                    | 663.655 | 0.000  |
| Female                  | 2260 (43.0)                                 | 508 (43.5)                                    |      |         |
| Positive Blood cultures | 515 (9.8)                                   | 536 (45.9)                                    | 913.275 | 0.000  |
| Negative Blood cultures | 4761 (90.2)                                 | 633 (54.1)                                    |      |         |
| Gram positive Bacteria  | 172 (33.3)                                  | 182 (34.0)                                    | 0.037 | 0.848   |
| Gram negative Bacteria  | 343 (66.7%)                                 | 354 (66.0)                                    |      |         |
| Positive culture        |                                             |                                               |      |         |
| Males                   | 287 (57.0)                                  | 286 (53.8)                                    | 1.062 | 0.303   |
| Females                 | 217 (43.0)                                  | 246 (48.2)                                    |      |         |
| Age                     |                                             |                                               |      |         |
| <28 days                |                                             |                                               |      |         |
| Positive Culture        | 282 (13.3)                                  | 219 (31.1)                                    | 424.247 | 0.000  |
| Negative culture        | 1833 (86.6)                                 | 144 (68.9)                                    |      |         |
| 1 - 12 mths             |                                             |                                               |      |         |
| Positive Culture        | 70 (7.2)                                    | 86 (17.5)                                     | 178.588 | 0.000  |
| Negative Culture        | 900 (92.8)                                  | 118 (82.5)                                    |      |         |
| >1 - 5 yrs              |                                             |                                               |      |         |
| Positive Culture        | 96 (8.2)                                    | 134 (26.2)                                    | 233.244 | 0.000  |
| Negative Culture        | 1073 (91.8)                                 | 172 (73.8)                                    |      |         |
| 6 - 9 yrs               |                                             |                                               |      |         |
| Positive Culture        | 44 (8.0)                                    | 41 (10.1)                                     | 62.581 | 0.000   |
| Negative Culture        | 506 (92.0)                                  | 77 (89.9)                                     |      |         |
| 10 - 18 yrs             |                                             |                                               |      |         |
| Positive Culture        | 23 (6.1)                                    | 56 (31.4)                                     | 63.928 | 0.000   |
| Negative Culture        | 355 (93.9)                                  | 122 (68.6)                                    |      |         |

common in this age group.
Staph aureus and klebsiella were the leading isolates by both culture technique in infancy and under 5 age group. Citrobacter, Enterococcus and pseudomonas were isolated by ABCS in addition. There were more contaminants in the younger than the older children. Table 3 shows the distribution of other isolates in other age groups. Overall, many more children had received a blood culture test by the manual than by the automated method.
Table 2. All Blood culture isolates in children 0 - 18 years.

| Blood Isolate                     | Conventional methods N (%) | Automated blood culture system N (%) |
|-----------------------------------|-----------------------------|--------------------------------------|
| **Klebsiella**                    | 128 (24.8)                  | 36 (7.3)                             |
| **Staphylococcus aureus**         | 114 (22.1)                  | 301 (61.2)                           |
| **E. coli**                       | 46 (8.9)                    | 11 (2.2)                             |
| **Enterococcus**                  | 21 (4.1)                    | 12 (2.4)                             |
| **Proteus spp.**                  | 17 (3.3)                    | 6 (1.2)                              |
| **Enterobacteriaceae**            | 17 (3.3)                    | 6 (1.2)                              |
| **Citrobacter**                   | 16 (3.1)                    | 41 (8.2)                             |
| **Pseudomonas**                   | 14 (2.7)                    | 29 (5.9)                             |
| **Salmonella**                    | 14 (2.7)                    | 2 (0.4)                              |
| **Providencia**                   | 14 (2.7)                    | 0 (0.0)                              |
| **Alcaligenes**                   | 13 (2.5)                    | 43 (8.7)                             |
| Alpha-Haemolytic streptococcus    | 13 (2.5)                    | 0 (0.0)                              |
| Beta-Haemolytic Streptococcus     | 4 (0.7)                     | 0 (0.0)                              |
| **Yersinia spp.**                 | 4 (0.7)                     | 0 (0.0)                              |
| **Shigella**                      | 3 (0.5)                     | 0 (0.0)                              |
| **Morganella**                    | 3 (0.5)                     | 0 (0.0)                              |
| **Serratia**                      | 2 (0.4)                     | 0 (0.0)                              |
| **Candida**                       | 2 (0.4)                     | 0 (0.0)                              |
| **Meningococcus**                 | 1 (0.1)                     | 0 (0.0)                              |
| **S. faecalis**                   | 1 (0.1)                     | 2 (0.4)                              |
| **Haemophilus**                   | 1 (0.1)                     | 0 (0.0)                              |
| **Contaminants**                  | 65 (12.6)                   | 0 (0.0)                              |

Table 3. Blood Culture Isolates and Age group: Manual (2008-2012) Vs Automated blood culture Systems (2015-2020).

| Pathogen                        | <28 days Manual | <28 days ABCS | >28 dy - 1 yr Manual | >28 dy - 1 yr ABCS | >1 - 5 yrs Manual | >1 - 5 yrs ABCS | >6 - 9 yrs Manual | >6 - 9 yrs ABCS | >10 - 18 yrs Manual | >10 - 18 yrs ABCS |
|---------------------------------|-----------------|---------------|----------------------|--------------------|------------------|-----------------|-----------------|-----------------|-------------------|------------------|
| **Klebsiella**                  | 104 (38.1)      | 17 (8.2)      | 6 (9.2)              | 3 (4.3)            | 11 (11.9)        | 10 (8.1)        | 3 (7.3)         | 2 (5.7)         | 2 (8.6)           | 4 (7.5)           |
| **S. aureus**                   | 54 (19.8)       | 103 (49.5)    | 24 (37.0)            | 57 (81.4)          | 21 (22.8)        | 83 (67.0)       | 7 (17.1)        | 22 (62.9)       | 2 (8.6)           | 36 (67.9)         |
| **E. coli**                     | 27 (9.9)        | 6 (2.9)       | 5 (7.7)              | 0 (0.0)            | 5 (5.4)          | 4 (3.2)         | 6 (14.6)        | 0 (0.0)         | 3 (13.0)          | 1 (1.8)           |
| Enterococcus                    | 14 (5.1)        | 6 (2.9)       | 2 (3.0)              | 3 (4.3)            | 2 (2.1)          | 2 (1.6)         | 2 (4.8)         | 0 (0.0)         | 1 (4.3)           | 1 (1.8)           |
| Enterobacter                    | 6 (2.2)         | 0 (0.0)       | 5 (7.7)              | 0 (0.0)            | 4 (4.3)          | 0 (0.0)         | 1 (2.4)         | 0 (0.0)         | 1 (4.3)           | 0 (0.0)           |
| **Alcaligenes**                 | 5 (1.8)         | 34 (16.3)     | 1 (1.5)              | 0 (0.0)            | 2 (2.1)          | 5 (4.0)         | 3 (7.3)         | 1 (2.8)         | 2 (8.6)           | 3 (5.6)           |
| **Citrobacter**                 | 4 (1.5)         | 22 (10.6)     | 1 (1.5)              | 4 (5.7)            | 8 (8.7)          | 8 (6.4)         | 3 (7.3)         | 6 (17.1)        | 0 (0.0)           | 2 (3.7)           |
| **Pseudomonas**                 | 6 (2.2)         | 12 (5.8)      | 3 (4.6)              | 1 (1.4)            | 3 (3.3)          | 8 (6.4)         | 2 (4.8)         | 4 (11.4)        | 0 (0.0)           | 4 (7.5)           |
### 4. Discussion

We report blood culture positivity rates of 45.9% by ABCS compared to 9.8% by conventional method in children 0 - 18 years in our facility. Earlier Reports [11][12][13] and studies [14][15][16] from developing countries showed that automated systems show better performance than manual systems in terms of yield, sensitivity and especially speed of growth. While there is paucity of similar studies in Nigeria, Medugu et al. [18] reported blood culture positivity rate of 32.7% by Bactec and 22% by the manual blood culture methods in children, even though this was not statistically significant. This might be attributed to their small sample size compared to this study. A greater pathogen yield was demonstrated by Bose et al. [22] in India with Bactec 24.1% and CM of 17.9% while 34% by Bactec and 24% by CM in was reported Pakistan [16]. While numerous factors such as prior antibiotic use, blood volume, blood stream infection periodicity, causative organism, bacteria density in the bloodstream, contribute to Blood Culture sensitivity, antibiotics prior to BC sampling decreases the rate of culture positivity by 45% - 50% [10][23][24].

This study did not report prior antibiotic use before blood culture, but the studies of Brown [25], Uzodinma [20] and obaro [26] in Nigeria, and reports from Ghana [27], Gambia [28] showed high levels of antibiotic use before culture. Over the-counter antibiotic use is very prevalent and this is likely to impact the outcome of any study aimed at the determination of the causes of bacterial infection in children. It is possible that this healthcare seeking behavior may on itself modify the spectrum of prevalent bacteria pathogens and the overall yield [26].
There was wide disparity in the positive culture yield between the two culture methods, and was statistically significant. Automated blood culture systems support the growth of a wider range of organisms and at lower inoculum than manual systems, they also have antibiotic removal devices such as resins which help to enhance microbial growth in the presence of antibiotics, the continuous agitation of bottles by the equipment also encourages bacterial growth [18] [29] [30]. This might have played a role in the better yield of Bactec as compared to CM in this study.

Timely and early determination of BSI especially in children is both a clinical and microbiologic imperative. While this study did not report time to identification of bacteria in Bactec, reports demonstrated shorter time to positivity by Automated systems over convectional method. Ahmad et al. [16] reported first positive blood culture results as early as six hours with automated blood culture system and the mean time to positivity for all cultures was 21 hours compared to 48 Hrs for CM to first positive blood culture. Similarly, Bose et al. [22] reported average time of growth of microorganisms of 24 hours in conventional system and 18 hours in automated system and Udayan and Dias [31], showed an average time taken for growth in a conventional method was 26.5 h, while that in BACTEC™ is 14.25 h. In Iran Alizadeh et al. [32] reported a rate of positive blood cultures employing BACTEC method of 100% while in the conventional method of 59.09%. Early commencement of appropriate antibiotics prevents antibiotic empiricism a major factor in antimicrobial resistance; it also mitigates hospitalization costs with cost savings in health. As survival of BSIs is inversely related with time to adequate antibiotic therapy, it is also important that results of blood cultures are available as soon as possible [6] [7] [8].

In both culture techniques, more males had positive blood cultures than females. This is similar with studies from Pakistan [16] and Nigeria [19]. There are reports of significant association between male gender and the development of community- and healthcare-associated BSI [33] [34]. The precise mechanisms by which gender might influence infection risk are unclear, but could possibly be related to differences in skin colonization or unknown anatomical differences between men and women [33].

The dominance of Gram-negative bacteria in both culture methods in this study is in consonance with reports from Nigeria [21] [35], South Africa [36], Tanzania [37], Ethiopia [38], Rwanda [39], India [40] [41] and Afghanistan [42] but in contrast to studies from Ghana [28] and the Gambia [29], where gram positive pathogens dominated in Children. Different age categories, variation in sampling and sample size, inadequate specimen volume, antibiotic exposure before culture and gaps in laboratory quality management in the Blood culture process may account for this finding [43] [44]. Data from Nigeria on the etiologic agents of bacteremia in children have been largely based on outdated blood culture methods, thus posing a challenge for data comparison [45].

Modern automated BC systems are highly effective at identifying the most
prevalent bacterial bloodstream pathogens. *Staphylococcus aureus* was the predominant bacteria isolated by the automated method in children in this study. This is in agreement with similar studies in Nigeria from Abuja [19], Lagos [20], Kano [46], Ibadan [47] [48] Ife [49] Maiduguri [50] using Bactec BD and with reports from Gambia [28] Uganda [51], Ethiopia [36], Ghana [28] [52], Guinea-Bissau [53] Pakistan [16] and Nepal [54]. It has become a leading cause of hospital and community-acquired bacteremia in children and is often used as a marker of invasiveness [55]. While in high-income countries, *S. aureus* bacteremia is the second most common cause of neonatal sepsis, after group B Streptococcus [56], in Africa, it is a common cause of invasive bacterial disease in children [19] [28] [38] [51] [52] [53].

*Alcaligenes* was the second most isolated pathogen by the automated blood culture method in this study. In Blood culture reports from Benin [57] and Abuja [58] a decade earlier using manual methods, *Alcaligenes* constituted 4.3% and 2% of isolates respectively. This uncommon non fermentative, gram-negative bacillus is a potentially emerging pathogen and usually causes opportunistic infections in humans [59]. The most commonly reported cases involved bacteremia, and most cases occurred in newborns and infants. They are generally considered as pathogenic than contaminants and misidentification of *Acinetobacter baumannii* as *Alcaligenes faecalis* by VITEK II system was reported in the literature. This differentiation from other oxidase-positive gram-negative rods may be difficult. This differentiation can be accurately done by the semiautomated systems now available years [60] [61] [62]. This easier identification systems may have contributed to the increasing reports of *Alcaligenes* spp in recent years.

In this study *Citrobacter* constituted the third most common isolate by Bactec method with greater detection rate over the manual method. These gram-negative bacilli constituted 1.3% [50], 1.9% [58] of blood cultures isolates in Nigeria studies; 0.3% in Rwanda [39] and Uganda [51] 3% in Ethiopia [63] and 15% in Ghana [27]. While methodologic variations may account for these differences, *Citrobacter* spp are opportunistic pathogens in humans that can lead to invasive disease, with sepsis and meningitis as the most common clinical manifestation in neonates and infants [64]. While the Bactec BD automated blood culture systems are effective in identifying polymicrobial infections in blood stream [65] [66], this and several studies [19] [20] [21] [22] [23] reported monomicrobial blood culture growth. The prevalence of PBSI is reported to be around 5% to 27% of all bloodstream infections in adult or pediatric patients [67].

The lower contamination rate in Bactec era compared to the Manual blood culture method may have been due the overall improvement in the microbiological environment with the establishment of multidisciplinary infection control and prevention committee in the hospital, regular bacterial surveillance of staff and equipment, antisepsis in blood culture sampling and formation of an infectious disease technical working group. There was 5-fold decline in blood culture in children after the introduction of the Bactec BD system in our facility. These
automated systems are costly, require regular maintenance and are not adapted to tropical, dusty environments, transferring costs to patients impeding the sustainable implementation of this technique in many developing countries [6][32]. If according to a market forecasting study, manual blood culture systems will make up roughly two-thirds of the global blood culture market by 2025 [68] recommendations for improvement in manual blood culture and clinical laboratory methods in low resource settings require urgency of implementation [6][69][70][71].

The absence of a nationally representative bacterial/antimicrobial surveillance system and weak microbiological diagnostic infrastructure, including coordination and management is stifling research and development with significant implications for public health and safety. This is therefore an urgent priority item on the agenda of health within the arm bit of one health in the country.

5. Conclusion

Blood culture yield by BACTEC automated system was about 5-fold greater than the conventional culture method in this study. Its cost has preluded many children from conducting this very critical test for Blood stream infection in our facility.

Limitation of the Study

Single blood culture bottle was used and prior antibiotic use before sampling blood for culture was not established. Disaggregation of children by disease diagnosis was not possible and time to culture positivity after incubation could not also be reported.

Recommendation

Establishment of nationally representative well-coordinated microbiologic diagnostic and surveillance laboratories in the country.

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Author Contribution

WEI and IJ Conceived of the study and study design, developed the first manuscript draft, and critically reviewed all drafts of the manuscript.

MM and IM critically reviewed bacterial isolates and reviewed draft manuscript.

AJD and CO Conducted quantitative analysis and critically reviewed the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.
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