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Molecular Detection of Pediatric Sepsis Pathogens and Antimicrobial Susceptibility to Antibiotics and *Leptadenia hastata* Extracts at Federal Medical Center Azare, Nigeria

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Abstract

Introduction: Sepsis is a significant public health issue causing high mortality rates in children, with Nigeria being the third-highest country globally. This study aimed to identify the bacterial pathogens responsible for sepsis in children visiting Federal Medical Centre Azare and determine their antimicrobial susceptibility profile for commercially manufactured antibiotics and extracts from *Leptadenia hastata*.

Methods: Blood samples were collected from children diagnosed with sepsis infections, cultured, and underwent genomic DNA extraction. PCR was conducted to target the 16S rRNA gene, and the bacterial isolates were subjected to sequence analysis and antibiotic susceptibility testing using the Kirby-Bauer disc diffusion method. *L. hastata* leaf extracts' effectiveness in inhibiting the isolates' growth was evaluated.

Result: The study found that *Streptococcus aureus* was the most prevalent isolate, followed by *Escherichia coli* 24 (24.7%), *Streptococcus pneumonia* 15 (15.4%), and *Salmonella* spp (3.2%). Sepsis was strongly correlated with age, with children under one month old being fourfold more likely to develop it [COR= 4.28, 95 CI, (1.746-10.493)]. Ceftriaxone, Augmentin, and Gentamicin were the best drugs for sepsis treatment, while 500mg of the plant extract showed the highest activity in ethanol, methanol, and aqueous.

Conclusion: The study found that Gram-positive bacteria were most prevalent and resistant to multiple drugs. The research contributed to identifying *L. hastata*, which can be used to control infections caused by bacteria resistant to antimicrobial infection, as it has higher efficacy against these isolates. It is necessary to regularly monitor the causative agent responsible for sepsis and their susceptibility to medicines.

Keywords: *Sepsis, Antimicrobial susceptibility test, Leptedenia hastata, PCR.*

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Introduction

Sepsis is a systemic illness caused by toxins producing bacteria invading the circulation from a local infection site (Lorke, 1983). Sepsis is mostly a community-acquired infection in Sub-Saharan Africa, with a poor prognosis due to the lack of obvious definite signs and symptoms in some cases (Morton *et al*., 2018). Time, etiological agent, geographic location, and antibiotic sensitivity pattern all played a role in the occurrence of sepsis (Weinstein and Doern, 2011). Those elements that influence test results, including the patient's age, underlying clinical problems, and etiologic agents, can be considered in the clinical diagnosis of sepsis (Gotts and Matthay, 2016).

A systematic review conducted in 2016 in highly developed nations revealed that there are more than 30 million instances of sepsis treated in hospitals worldwide each year, leading to 5.3 million fatalities attributable to sepsis (Fleischmann *et al*., 2016). There are noticeable variations in the prevalence of sepsis across different regions (Sakr *et al*., 2018). A study conducted in China found that sepsis cases requiring hospitalization increased from 328.25 to 421.85 per 100,000

people annually between 2017 and 2019. Based on these discoveries, the World Health Organisation (WHO) has officially designated sepsis as a worldwide health concern (Organization, 2020). Sepsis is responsible for over 30% of mortality in Nigeria, making it the highest in Africa and third in the world behind India and China. Children as young as 12 years old have been included in most sepsis studies (Uzodimma *et al*., 2013).

Although sepsis is a manageable, it is often exacerbated by antimicrobial resistance (AMR), leading to extended hospital stays and potentially lethal consequences (Goldstein *et al*., 2019). Antibiotic resistance worldwide is a growing concern, with the World Health Organization (WHO) recognizing it as a significant global threat in 2015 and the United Nations recognizing it in 2019 (Organization, 2021). The growing antibiotic resistance issue prompts a focus on discovering biologically active chemicals in plant species used in herbal medicine. *Leptadenia hastata*, a desirable herbaceous plant with creeping latex stems and smooth foliage, is grown in arid tropical regions with sandy soil. *L. hastata* is used in Nigeria as a spice, vegetable, and healer for high blood pressure and dermatological conditions (Ibrahim *et al*., 2012, Dambatta and Aliyu, 2011). It has also been found to have antifertility effects in West African breeders (Arbonnier, 2009). The current study aimed to detect sepsis bacterial etiology and antibiotic susceptibility patterns using commercial antibiotics and *L. hastata* leaf extract among children at Federal Medical Center Azare, Nigeria.

Materials and Method

Ethical Consideration and Consent Participation

The study received approval from the ethical review boards of Bauchi State University and Federal Medical Centre Azare, Bauchi, Nigeria. Participants were informed about the study's objectives and methods, and written informed consent was obtained. The collected data and samples were kept confidential and used solely for research purposes. Participant information was anonymized to ensure privacy. If positive results were obtained, the researchers collaborated with the attending physician to ensure proper treatment.

Study Design and Area

This research is a cross-sectional investigation carried out from August 2023 to January 2024 among children admitted to FMC Azare. Federal Medical Center (FMC) is a tertiary hospital owned by the Federal Government of Nigeria. FMC Azare is a tertiary care hospital that provides specialized treatments, including the management of sepsis and other infectious diseases, serving patients across Bauchi State and neighboring regions.

Study Population

The study involved children suspected of sepsis from the paediatrics department of Federal Medical Center Azare, Bauchi State, who had at least two symptoms (fever, chills, dizziness, difficulty breathing, low blood pressure, fast heart rate) were included in the study. A simple random sampling technique was used to select children suspected of sepsis who fulfills the inclusion criteria.

Sample Size Determination

The necessary sample size was determined using a formula for a single population proportion. A prevalence rate of 34.4% was obtained from a prior study conducted among newborns with sepsis in North Central Nigeria (Onyedibe *et al*., 2012), which was used to determine the sample size.

$$N = \frac{Z^2(p(1-p))}{d^2}$$

According to a prior study, the expected prevalence is 34.4% (0.34) with a precision or margin of error of 5% (d = 0.05). Z is equal to 1.96, and N represents the sample size. A minimum sample size of 345 was determined. Nevertheless, an additional 5% was included to account for non-respondents, resulting in a total sample size of 368.

Samples/Data Collection

Blood samples were collected from patients using a 5ml sterile syringe and transferred to a McCartney bottle containing fluid thioglycollate medium. Each bottle was marked with the patient's unique identifier and collection date. The samples were delivered to the laboratory within 30 minutes, and socio-demographic data was collected using a semi-structured pretested questionnaire.

Blood Culture

Blood samples were incubated at 37°C for a week to detect microbial growth. Observable signs include deposits on the liquid's surface, cloudiness, hemolysis, film formation, gas production, or liquid coagulation. A sub-culture was conducted when microbial proliferation was detected in a blood culture bottle.

Blood Sub-culture

Blood cultures exhibiting microbial growth were sub-cultured on blood agar, MacConkey agar, and chocolate agar plates using a direct streaking method. Blood and MacConkey plates were incubated in aerobic conditions, while chocolate plates were placed in an anaerobic environment. The plates were kept at 37 º C for 24 hours to examine bacteria growth, and cultures were assessed as negative or positive.

Identification of Bacterial Pathogens

The bacteria isolated from subculture plates were grown as pure cultures and tested for pathogen identification. Different biochemical tests were used for Gram-negative organisms, such as indole, carbohydrate fermentation, citrate utilization, urease, motility, lysine decarboxylase, lysine deaminase, and oxidase test, Gram-positive bacteria, catalase, coagulase, and mannitol fermentation, following the methods according to CLSI (Wayne, 2015).

Genomic DNA extraction and PCR Amplification of 16S rRNA gene

The genomic DNA was extracted from samples and purified using the Wizard Genomic DNA Purification Kit following the directions provided by the manufacturers. The 16S rRNA gene was amplified using universal primers E16S-F and E16S-R. The primer sequence consists of E16S-F (5′-CCCCCTGGACGAAGACTGAC-3′) and E16S-R (5′-ACCGCTGGCAACAAAGGATA-3′) (Wang *et al*., 2002).

The PCR reaction was conducted in a 50µL solution with nuclease-free water, DNA template, EconoTaq® PLUS 2X Master Mix, and primers. The process began with an initial denaturation phase at a temperature of 94℃ for 3 minutes. This was followed by 35 cycles of amplification, which involved denaturation at 94℃ for 10 seconds, annealing at 61℃ for 30 seconds, and extension at 72℃ for 1 minute. The process was concluded with a five-minute final extension at a temperature of 72℃. The PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, USA) following the manufacturer's instructions.

Sequence Analysis and construction of Phylogenetic tree

The 16S rRNA gene sequencing results were confirmed using BLAST software in the NCBI GenBank database, and the sequences were aligned using Clustal W tools in Version 7.0 MEGA Analysis Software. A phylogenetic tree was constructed using the neighbour-joining tree method in MEGAX software.

Antibiotic Susceptibility Pattern of the Isolates

The antibiotic susceptibility of Gram-positive and Gram-negative bacterial isolates was assessed using the Kirby-Bauer disk-diffusion method, as outlined by the Clinical and Laboratory Standards Institute (CLSI) guidelines (Wayne, 2015). Briefly, pure colonies were suspended in 5 ml of sterile normal saline to create a solution corresponding to a 0.5 McFarland standard. An aseptic technique was employed to collect samples from the suspension and evenly spread them onto Mueller-Hinton agar (MHA) using a sterile cotton swab. Seven antibiotics, namely, ceftriaxone: (Rocephin) (30 µg), gentamicin: (Gentamicin sulfate), augmentin (Amoxicillin/clavulanate acid) (5 µg), ofloxacin (OFX), erythromycin (15 µg), ceftazidime: (Ceftazidime pentahydrate), cefuroxime: (Cefuroxime axetil) (15 µg), and cloxacillin: (Cloxacillin sodium) (10 µg), were applied to the MHA plates. The plates were then incubated at 37℃ for 16-18 hours. The results were interpreted by assessing the zone of inhibition as susceptible (S), intermediate (I), or resistant (R) following the standardized CLSI guidelines (Wayne, 2015).

Collection and Authentication of the Plant

The leaves of *L. hastata* were collected and authenticated at Bauchi State University Gadau's Biological Sciences Department, then cleansed, dried, and pulverized into a fine powder in a controlled laboratory environment, which was then stored at ambient temperature.

Preparation of Aqueous, Methanol, and Ethanol extract

Preparing *L. hastata* extracts involves aqueous, methanol, and ethanol extract. Aqueous extracts are prepared by soaking powdered plant material in distilled water and stored at ambient temperature for three days. Filtration is done using Whatman filter paper, and the extract is evaporated at 70℃ in a water bath until completely dried. The desiccated extract samples are diluted in 30% *Dimethyl sulfoxide* (DMSO) to achieve a final 500 mg/mL concentration. Various concentrations of 350 mg/mL, 200 mg/mL, 100 mg/mL, and 50 mg/mL are prepared using the original stock concentration. Methanol extracts are obtained by immersing 50g of plant material in 500 ml of methanol and storing it at room temperature for three days. The extract evaporates at 60℃ in a water bath until completely dried. The dried extract samples are dissolved separately in 30% DMSO for a final 500 mg/mL stock concentration. Various concentrations of 350 mg/mL, 200 mg/mL, 100 mg/mL, and 50 mg/mL are prepared using the original stock concentration.

Ethanol extracts are obtained by immersing 50g of plant material in 500 ml of ethanol and storing it in a shaking machine for three days. The desiccated extract samples are dissolved individually in 30% DMSO to achieve an ultimate stock concentration of 500 mg/mL. Various concentrations of 350 mg/mL, 200 mg/mL, 100 mg/mL, and 50 mg/mL are prepared using the original stock concentration (Wayne, 2015).

Disc Diffusion

The antibacterial activity of isolates was assessed using the disc diffusion method. A concentrated solution of 0.5g of extract was dissolved in DMSO, resulting in a 500 mg/L concentration. Mueller Hinton agar plates were prepared, and the test microorganisms were calibrated. The microbial suspension was evenly distributed across the plates, and test extracts were applied to paper discs. The discs were left undisturbed for 5 hours, then incubated at 37℃ for 18-24 hours. The inhibition areas were quantified, including the zone of inhibition caused by augmentin as a positive control (Wayne, 2015).

Statistical Analysis

The data analysis was performed utilizing the SPSS version 27.0 software package. A chi-square test and bivariate logistic regression were conducted to investigate potential relationships between the independent variables and the outcome variable, sepsis. The association between independent variables and the outcome variable was evaluated by calculating crude odds ratios and 95% confidence intervals to determine the strength of the relationship.

Results

The study showed that out of the 368 culture results, 97(26.4%) were culture-positive. Of the patients enrolled, 198 (53.8%) were male, and 170 (46.2%) were female. The highest proportion was observed in the male, 52 (26.3%), while the female had 45 (26.5%). The observed differences in the proportion among sexes do not vary significantly (χ^2 =0.001, df = 1, p = 0.964) as shown in Table 1. The bivariate analysis indicated that the age group under one month had a statistically significant correlation with sepsis, as they were almost fourfold more prone to developing sepsis than their counterparts [COR= 4.28, 95% CI, (1.746-10.493)]. Participants aged one month to 1 year and 2-5 years exhibited a statistically significant association with sepsis. They were approximately two times more likely to have sepsis compared to their counterparts aged 6-10 years [COR= 2.80, 95 CI, (1.242-4.486)], and [COR=2.31, 95 CI, (1.243-4.300)] (Table 1).

Bacteriological profiles of culture-positive patients.

Table 2 shows that 97 bacterial pathogens were found among 368 blood culture sets. Out of the isolates responsible for

sepsis, the majority, specifically 50 out of 97 (51.4%), were Gram-positive bacteria such as *S. aureus* and *S. pneumonia*. On the other hand, Gram-negative bacteria included *Klebsiella* species, *E. coli*, *K. pneumonia*, *P. aeruginosa*, *Enterobacter species*, and *S. typhi*, accounting for 47 out of 97 (48.6%) isolates. Among the Gram-positive bacteria, *S. aureus* 35/97 (36.0%) was the most common, followed by *S. pneumonia* 15/97 (15.4%). The most frequently obtained Gram-negative bacteria was *E. coli*, with 24/97 (24.7%) cases, followed by *K. pneumonia* 9/97 (9.3%), *P. aeruginosa* 7/97 (7.2%), *Enterobacter* species 4/97 (4.2%), and *S. typhi* 3/97 (3.2%).

Furthermore, the 16S rRNA gene was used to identify bacterial pathogens through a molecular approach, resulting in a single band, as shown in Figure 1. The 16S rRNA gene sequence was compared with reference sequences in the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) to identify the precise bacterial species. A phylogenetic tree was then constructed for *S. aureus* using available reference sequences in NCBI. Upon analysis, it was found that the isolated *S. aureus* showed 100% homology with other *S. aureus* strains present in the NCBI database (Figure 2).

Table 1: Socio-demographic and Bivariate logistic regression analyses of sepsis-suspected patients attending FMC Azare, Northeast Nigeria.

Variables	Frequency	No. positives (%)	χ^2	p-value	COR	p-value
Sex						
Male	198 (53.8)	52 (26.3)	0.002	0.964	0.989 (0.621-1.575)	0.964
Female	170 (46.2)	45 (26.5)			1	
Age						
<1 Month	27 (7.4)	12 (44.4)	13.699	0.003	4.280 (1.746-10.493)	0.001
1 month- 1year	98 (26.6)	30 (30.6)			2.804 (1.242-4.486)	0.009
2 years – 5 years	116 (31.5)	35 (30.2)			2.312 (1.243-4.300)	0.008
6 years -10 years	127 (34.5)	20 (15.7)			1	

Table 2: Bacterial isolates of blood culture among patients attending FMC, Azare, Northeast Nigeria.

Gram-reaction	Isolated species	Frequency	Percentage (%)
Gram-positive bacteria	*S. aureus*	35	36.0
	S. pneumonia	15	15.4
Gram-negative bacteria	*E. coli*	24	24.7
	K. pneumonia	9	9.3
	p. aeruginosa	7	7.2
	Enterobacter species	4	4.2
	S. typhi	3	3.2

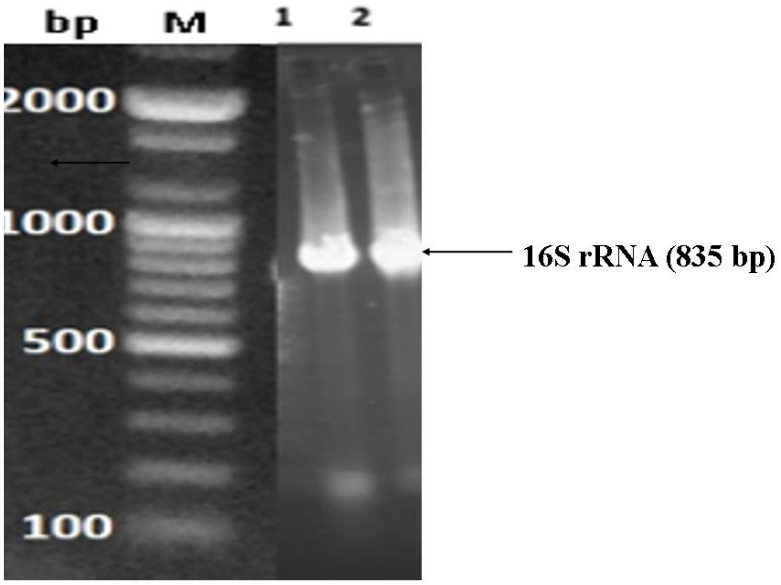

Figure 1: Agarose gel electrophoresis of amplified 16S rRNA gene using universal primer. Lane: M-represents marker, lanes 1-2 represent isolates of *S. aureus*

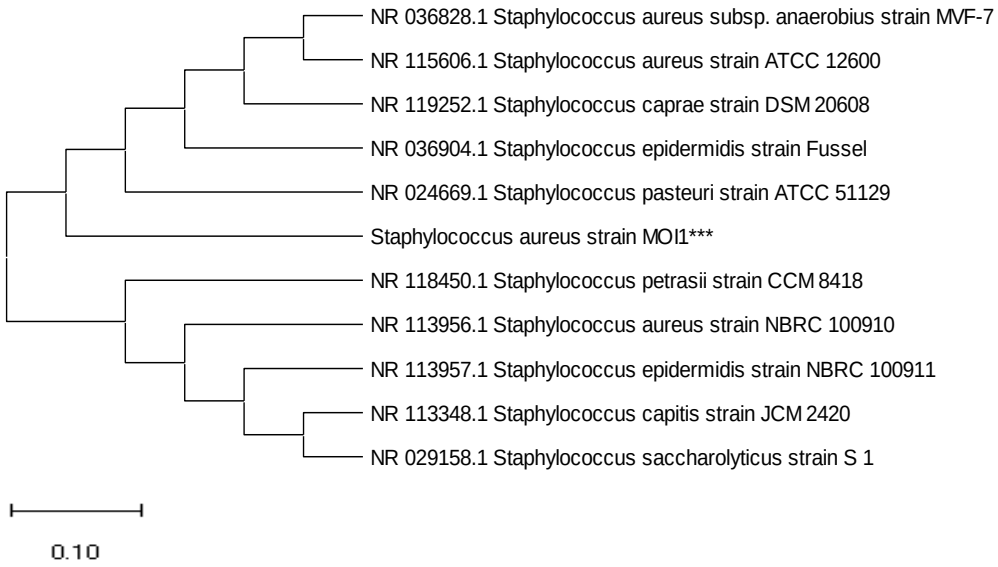

Figure 2: A phylogenetic tree showing the relationship between the 16S rRNA gene sequences of Isolated S. aureus and that of other *S. aureus* species from NCBI. The *S. aureus* isolated was designated as *S. aureus* strain MOI***. The Evolutionary relationship was inferred using the Neighbor-joining method. The horizontal bar indicates 0.10.

Antibiotic Susceptibility Pattern of Pathogenic Bacteria Associated with Sepsis Infection

In the treatment and management of infections, the effectiveness of antibiotics is a critical factor. Our study focused on determining the efficacy of various antibiotics against sepsis infections caused by Gram-positive and Gram-negative bacteria. Figures 3 and 4 present our findings.

We tested *S. aureus* for Gram-positive bacteria. Ceftriaxone (90%), augmentin (77%), and ofloxacin (71%) showed sensitivity to *Staphylococcus aureus*, while ceftazidime (60%), cefuroxime (57%), and cloxacillin (71%), erythromycin (63%), and gentamicin (54%) showed resistance. For Gram-negative bacteria, we tested *E. coli*. The results showed high sensitivity to ceftriaxone (92%), augmentin (100%), and ofloxacin (88%). However, it exhibited maximum resistance to ceftazidime (60%), cefuroxime (67%), cloxacillin (58%), erythromycin (54%), and gentamicin (63%).

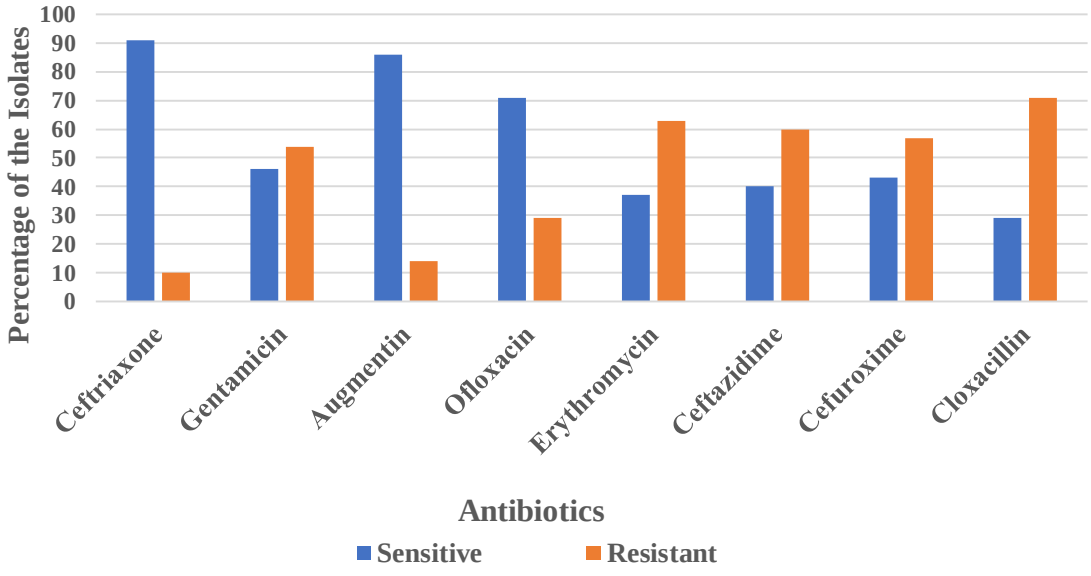

Figure 3: Antibiotics susceptibility pattern of *S. aureus* recovered from children with sepsis infection.

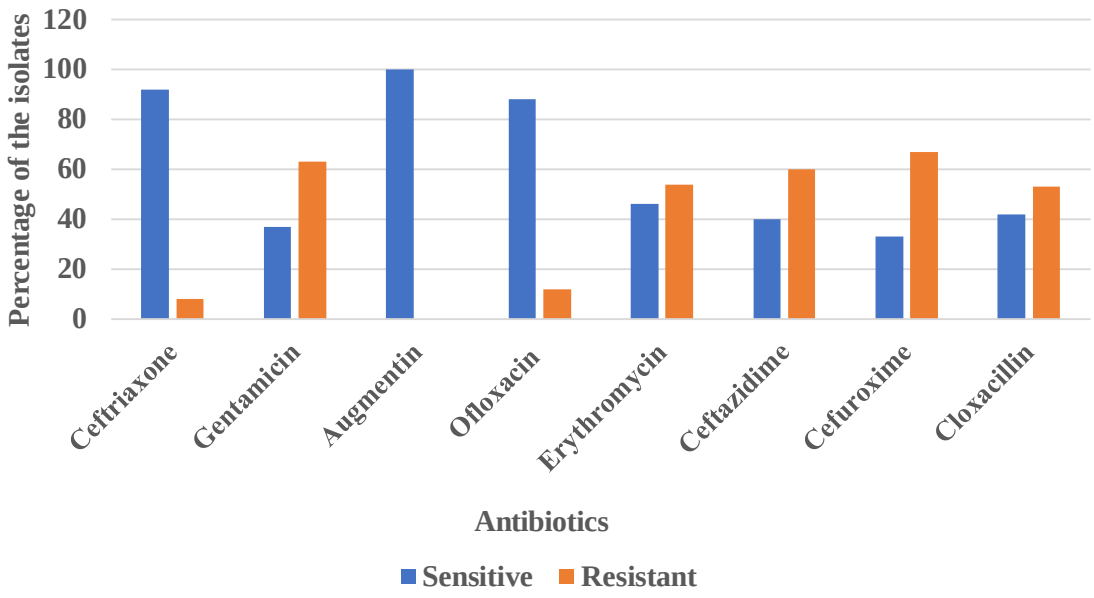

Figure 4: Antibiotics susceptibility pattern of *E. coli* recovered from children with sepsis infection

Antibacterial activities of *L. hastata* leaves extracts against *S. aureus, E. coli*, and *S. pneumoniae*

The present study examined the antibacterial effects of *L. hastata* on three types of bacteria (*S. aureus*, *E. coli*, and *S. pneumoniae*) using three distinct extracts (methanolic, ethanolic, and aqueous). The findings were analyzed and presented in Table 3. Each extract demonstrated a higher inhibition rate as the concentration increased. Notably, the methanolic extract produced the greatest growth inhibition zone, reaching 21.0, 23.2, and 20.0 for the three bacteria at a 500mg/ml concentration. The ethanolic and aqueous extracts also demonstrated inhibition at this same concentration, with a zone of growth inhibition of 19.6, 22.4, and 20.0, and 19.8, 21.3, and 19.5, respectively. Intermediate inhibition was observed for all extracts at 350mg/ml, while no significant zone of inhibition was observed at 200mg/ml, 100mg/ml, and 50mg/ml. Although all three extracts showed activity at 500mg/ml, the methanolic extract proved to be the most effective with a wider zone of inhibition.

Table 3: Antibacterial activities of methanolic, ethanol, and aqueous leaves extract of *L. hastata*

Zone of growth inhibition (mm)	**Test organism**		
Methanol Extract	***S. aureus***	***E. coli***	***S. pneumoniae***
500	21.0 ± 0.82	23.2 ± 1.26	23.2 ± 1.26
350	15.7 ± 0.47	17.7 ± 0.48	17.3 ± 0.48
200	12.3 ± 0.46	14.0 ± 0.82	12.3 ± 0.47
100	8.30 ± 0.47	8.60 ± 0.47	7.00 ± 0.45
50	0	0	0
Augmentin (30µg)	24	24	24
Ethanol Extract			
500	19.6 ± 0.47	22.4 ± 0.49	20.0 ± 0.82
350	14.4 ± 0.47	19.0 ± 0.81	13.0 ± 0.81
200	11.0 ± 0.80	13.0 ± 0.81	11.0 ± 0.81
100	7.30 ± 0.44	8.50 ± 0.47	7.40 ± 0.45
50	0	0	0
Augmentin (30µg)	24	24	24
Aqueous Extract			
500	19.8 ± 0.48	21.3 ± 0.49	19.5 ± 0.48
350	18.0 ± 0.82	17.3 ± 0.46	14.0 ± 0.81
200	14.5 ± 0.46	13.0 ± 0.81	11.3 ± 0.49
100	8.30 ± 0.47	8.60 ± 0.47	7.00 ± 0.45
50	0	0	0
Augmentin (30µg)	24	24	24

Discussion

The prevalence of sepsis in the present study revealed 26.4%. The culture-positivity rate is comparable to the 23.9% and 26.7% found in southern Nigeria (Peterside *et al*., 2015, Ojukwu *et al*., 2006) and other parts of African countries such as Ghana (Acquah *et al*., 2013), which reported 25.9%. However, the prevalence is relatively higher than the 11.0% reported by Morkel *et al*. (2014) in South Africa. However, the prevalence obtained was lower than in some parts of Nigeria, such as Ilorin (Mokuolu *et al*., 2002), and other African countries, such as Zambia (Kabwe *et al*., 2016), which recorded a 33.0% prevalence of sepsis.

The differences in prevalence rates can be ascribed to the varied procedures employed for blood culture, which can be manual or automated. Other factors contributing to the variation include the quantity of blood sampled, the number of blood cultures collected, and the absence of clear clinical indicators. These characteristics led to a higher percentage of poor outcomes and higher prevalence rates (Lamy *et al*., 2016). Additionally, the size of the sample, the type of patients, the study's design, geographical locations, seasonal variation, the frequency of blood cultures, and the infection control practices used in various nations could all have played a role (Birru *et al*., 2021).

Our study revealed a higher incidence of sepsis among infants less than one month old (44.4%) compared to other age groups. This study has determined that the disease impacts individuals of all age groups. However, it was particularly prominent among infants less than one month old compared to other age groups, and this disparity is statistically significant (p=0.001). The binary logistic analysis revealed that infants under one month of age have a fourfold higher likelihood of developing sepsis compared to those aged 6-10. The elevated prevalence of sepsis among infants less than one month old in the study region can be attributed to their diminished immune response, the socioeconomic level of their parents, and inadequate hygiene practices.

Female individuals exhibit a slightly higher incidence of sepsis (26.5%) compared to their male counterparts (26.3%). Nevertheless, there was no statistically significant correlation observed between gender and sepsis in the pediatric population. This finding is consistent with the research conducted by Ali and Kebede (2008) in Ethiopia, which documented a significant incidence of sepsis among female children. However, this contradicts the findings of Komolafe and Adegoke (2008), who reported a substantial frequency of sepsis among males. The authors suggested that the higher occurrence in males could be linked to exposure factors and specific behavioural attitudes/activities, which render them more susceptible to accidents.

The causative pathogens of sepsis exhibit geographical variations, reflecting distinct characteristics in different countries (Birru *et al*., 2021). The predominant bacterial kinds that are frequently isolated belong to the Gram-positive group, accounting for 51.4% of the total, while their Gram-negative counterparts make up 48.4%. Previous studies conducted in various regions, including Bayelsa in Nigeria (Peterside *et al*., 2015) and Ethiopia (Wasihun *et al*., 2015), have also observed a similar predominance pattern displayed by the Gram-positive group. In Bayelsa, the prevalence was 58.1%, and in Ethiopia, 72.2%. However, this contradicts the findings of other studies conducted in Nigeria (Nwadioha *et al*., 2010) and Cameroun (Yimtchi *et al*., 2023), which identified Gram-negative bacteria as the predominant group. The differences in the occurrence and range of causative agents may have had a role in this discrepancy (Birru *et al*., 2021, Wasihun *et al*., 2015). The percentages may arise due to methodological differences, patient safety protocols, diagnostic methodologies, and sample sizes.

S. aureus was the predominant strain (36.0%) in the Gram-positive category in the blood culture. According to the current study, similar prevalence patterns have also been observed in Ethiopia (Ameya *et al*., 2020) and other parts of Nigeria, including Calabar (Meremikwu *et al*., 2005). In contrast to a study conducted in Ethiopia, the most prevalent bacteria found were Coagulase-negative staphylococci (CONS), then *S. aureus* (Dagnew *et al*., 2013). *S. aureus* has a high incidence due to its pervasive contamination of hospital surroundings, which may have invaded newly admitted patients and led to the development of infections (Birru *et al*., 2021). Additionally, it has the potential to penetrate the patient's body during surgery and other mechanical procedures due to its presence as a symbiotic organism on the skin and mucous membranes. Nevertheless, additional investigation is required before formulating any definitive inferences.

E. coli was the most prevalent Gram-negative bacteria isolated. A similar pattern was noted in prior research carried out in Nigeria (Peterside *et al*., 2015) as well as in other regions throughout the globe, including South Africa (Morkel *et al*., 2014) and India (Gupta and Kashyap, 2016). Contrary to our results, research conducted in Nigeria and Ethiopia has indicated that *K. pneumoniae* is the most prevalent strain, with *E. coli* following closely behind (Nwadioha *et al*., 2010, Dagnew *et al*., 2013). The findings of Khan *et al*. (2019) in Saudia Arabia agree with ours. The fact that many Gram-negative bacteria are the primary manufacturers of enzymes (ESBLs) that render medications with beta-lactam functional groups, such as cephalosporins, inactive also explains why so many of them are in the current study. The second most prevalent gram-negative bacteria in this study was *K. pneumonia*, which aligns with findings from prior studies conducted in India (Sahoo *et al*., 2016). Contrary to our results, a study conducted in Ethiopia (Birru *et al*., 2021) has indicated that *Klebsiella* spp is the most prevalent gram-negative pathogen among the often-encountered diseases. AMR poses a significant risk to human health worldwide (Murray *et al*., 2022). Prior research has assessed the influence of AMR on the occurrence, fatalities, duration of hospitalization, and expenses related to healthcare for particular combinations of pathogens and drugs in distinct geographical areas (Murray *et al*., 2022). The findings of our study indicate that ceftriaxone, augmentin, and ofloxacin have demonstrated efficacy against Gram-positive bacteria, such as *S. aureus*, and Gram-negative bacteria, particularly *E. coli*. These results are consistent with a recent study conducted by Omenako *et al*. (2022), which also demonstrated the efficacy of ceftriaxone and ofloxacin against

S. aureus and *E. coli*. However, our findings contradict those of Ameya *et al*. (2020), where augmentin and ceftriaxone were resistant to *E. coli*. Additionally, we discovered that gentamicin showed resistance to *E. coli*, which aligns with our findings.

Moreover, our study found that *S. aureus* and *E. coli* isolates were highly resistant to most antibiotics, including ceftazidime, cefuroxime, cloxacillin, erythromycin, and gentamicin. This finding is similar to the result of Pradipta *et al*. (2013), where all pathogens were resistant to six major antibiotics, including ceftriaxone. However, in our study, ceftriaxone was highly sensitive to all pathogens. Our finding agrees with the results of Legese *et al*. (2022), where two antibiotics, namely cefuroxime and ceftazidime, showed resistance to the isolated pathogens. Meanwhile, our result agrees with the report of Khan *et al*. (2023), where gentamicin was found resistant to *E. coli*. At the same time, it disagrees with our results, where ceftazidime was found to be sensitive.

According to various studies, the six most common pathogens that have caused deaths due to AMR are *E. coli, S. aureus, K. pneumoniae, S. pneumoniae, A. baumannii,* and *P. aeruginosa* (Murray *et al*., 2022). These pathogens were responsible for causing 929,000 deaths related to AMR, and a total of 3.57 million deaths were associated with AMR in 2019 (Murray *et al*., 2022). Multi-drug resistance occurs when various resistance mechanisms are simultaneously triggered in response to antibiotic exposure (Macias *et al*., 2022). Several processes contribute to this, such as the production of chromosomally encoded extended-spectrum beta-lactamase (ESBL), decreased permeability caused by the loss of porin channels, and the activation of multi-drug efflux pumps (Pop-Vicas and Opal, 2014). In addition, the transfer of plasmids and migratory elements that carry numerous resistance genes plays a role in developing characteristics that make bacteria resistant to several drugs. Controlling antibiotic resistance in gram-negative bacteria poses significant difficulties (Macias *et al*., 2022).

The antimicrobial activity of *L. hastata* against test organisms, such as *S. aureus, E. coli,* and *S. pneumonia*, was similar to the findings of previous studies conducted by Sani *et al*. (2021) in Nigeria. However, our results agree with the outcomes of studies conducted by Umaru *et al*. (2019) in Malaysia. On the other hand, our study's findings regarding the antimicrobial activity of *L. hastata* differ from the results of Mahamat *et al*. (2021), where the *L. hastata* extract exhibited only moderate activity against *S. aureus*.

Conclusion

The study findings indicate that the blood culture analysis revealed six pathogens: *S. aureus, E. coli, S. pneumoniae, Salmonella, P. aeruginosa*, and *Enterobacter* species. The prevalence of Gram-positive isolates, namely *S. aureus*, was the highest, but *E. coli* was the most common among Gram-negative isolates. Resistance to ceftazidime, cefuroxime, cloxacillin, erythromycin, and gentamicin was observed in both Gram-positive and Gram-negative isolates. Antibiotics ceftriaxone, augmentin, and ofloxacin were efficacious against all isolates. Children below one month of age have an increased susceptibility to the acquisition of sepsis infection. The leaf extract of *L. hastata* exhibited the highest antibacterial efficacy at a concentration of 500mg. Regular antibiotic susceptibility testing can effectively mitigate the development of drug resistance. Thus, our study encourages using *L. hastata* extracts as a therapeutic plant due to its significant antibacterial properties.

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