**CHARACTERIZATION AND ANTIBIOTIC RESISTANCE OF GRAM NEGATIVE BACTERIA INVOLVED IN SEPSIS AMONG UNDER FIVE CHILDREN IN AKWA IBOM STATE NIGERIA**

**ABSTRACT**

Aim: This study characterized Gram-negative bacteria, their antibiotic resistance, and associated resistance genes in sepsis among children under five in Akwa Ibom State, Nigeria.

Study Design: A hospital-based descriptive observational study of neonates with or without clinical features of sepsis.

Place and Duration: Conducted at General Hospital Ikot Ekpene, University Teaching Hospital Uyo, and Immanuel Hospital Eket between June 2023 and December 2024.

Methodology: A total of 180 children (0–5 years, both sexes) were included, with 60 participants from each hospital, recruited from outpatient, pediatric, gynecological, and immunization clinics. Blood samples (2 ml) were collected aseptically and cultured on thioglycollate broth, then subcultured on MacConkey, blood, and chocolate agar. Gram staining, biochemical characterization, and antimicrobial susceptibility testing were performed. Resistance genes were identified, and demographic, environmental, and social data were collected via caregiver interviews and structured questionnaires.

Results: Of the 180 children, 123 tested positive for bacterial infections, with *Escherichia coli* (13.9%), *Proteus mirabilis* (10.6%), *Pseudomonas aeruginosa* (8.3%), and *Klebsiella pneumoniae* (6.7%) being the most common. Risk factors for sepsis were not statistically significant (P > 0.05), except for location (P < 0.05), with Uyo Teaching Hospital showing higher significance. *P. aeruginosa* exhibited 100% resistance to all 12 tested antibiotics. Resistance gene analysis revealed that *E. coli*, *P. aeruginosa*, and *K. pneumoniae* harbored blaSHV, PAGS, PASS, Cnf1, and hlyC genes. *Pseudomonas* and *Klebsiella* carried the blaTEM gene, while only *Klebsiella* harbored FimH.

Conclusion: Gram-negative bacteria exhibit significant antibiotic resistance, posing challenges in managing infections in young children.

**Keywords:** Sepsis, Antibiotic, Resistance, Gram-Negative Bacteria, Children.

1. **INTRODUCTION**

**1.1 Background of the Study:** Sepsis is a life-threatening condition resulting from an uncontrolled immune response to infection, leading to systemic inflammation, organ failure, and death (Basco, 2021)1. It remains a major global health challenge, particularly among neonates and children under five, with Gram-negative bacteria being the predominant cause (Rudd *et al*., 2020)2. The rising prevalence of multidrug-resistant (MDR) Gram-negative pathogens, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, has significantly contributed to increased mortality rates, particularly in resource-limited settings like Nigeria. In Nigeria, neonatal sepsis incidence is alarmingly high Olorukooba *et al*., (2020)3, with mortality rates exacerbated by inadequate access to effective antimicrobial therapy. Despite WHO-recommended first-line treatments such as gentamicin and ampicillin, antimicrobial resistance (AMR) has severely compromised treatment efficacy. Identifying resistance patterns and the genetic mechanisms underlying AMR in Gram-negative sepsis is critical for improving diagnostic and therapeutic strategies. This study aims to characterize Gram-negative bacterial pathogens responsible for sepsis in children under five in Akwa Ibom State and assess their antibiotic resistance profiles and associated resistance genes. Findings from this research will contribute to better infection control, antimicrobial stewardship, and improved clinical outcomes for pediatric sepsis cases.

**2.0 MATERIALS AND METHODS**

**2.1 Study Area and Population:** This 18 month study (June 2023-December 2024) was conducted in three senatorial districts of Akwa Ibom State, Nigeria: Ikot Ekpene (North West), Eket (North South), and Uyo (North East). Three hospitals General Hospital Ikot Ekpene, Immanuel Hospital Eket and Teaching Hospital Uyo were selected. The study assessed all admitted children (0-5 years) with or without clinical signs of sepsis. Akwa Ibom, located in Nigeria’s south-south geopolitical zone, shares borders with Cross River, Rivers, and Abia States and the Atlantic Ocean. It has 31 local government areas, with Ibibio as the dominant language. The economy relies on trading, fishing, and palm oil exports. Study sites were selected for convenience and proximity to the laboratory for specimen analysis. The study population consisted of children (both male and female), children under five years of age (0-5) seen in general out-patient, gynaecological out-patient clinics, pediatric clinics and immunization unit in the hospital of study.



**Figure 1:** Map showing the three Senatorial Districts in Akwa Ibom State (NPC, 2011)4.

**2.2 Sample Collection and Processing:** A total of 180 blood samples were aseptically collected using sterile syringes and transferred to the clinical microbiology laboratory within 24 hours for culture. Gram negative bacteria were isolated using thioglycollate broth, incubated at 35-37°C for 7 days, and subcultured on selective media (MacConkey, Blood and Chocolate agar).

**2.3 Identification and Characterization:** Bacterial isolates were identified using Gram staining and the Biomerieux VITEK 2 system. Biochemical characterization followed standard protocols to confirm bacterial species.

**2.4 Antimicrobial Susceptibility Testing:** The Kirby-Bauer disc diffusion method was used to assess antibiotic susceptibility according to CLSI guidelines. Multidrug resistance (MDR) was determined using the multiple antibiotic resistances (MAR) index.

**2.5 Molecular Analysis:** DNA extraction was performed using the Zymo Research Quick-DNA Fungal and Bacteria Kit, followed by quantification using a spectrophotometer (Gene Quant Pro) at 260/280 nm.

 **Table 1: DNA Concentration and Purity of Bacteria Samples**

|  |  |  |
| --- | --- | --- |
|  **Sample ID** | **DNA Concentration (ug/ul)** | **DNA Purity (260/280)** |
|  **A** |  311 |  1.82 |
|  **B** |  395 |  1.89 |
|  **C** |  361 |  1.80 |

**2.5.1** **Gel Electrophoresis:** DNA quality was checked using 1.5% agarose gel electrophoresis in 1X TAE buffer at 120V for 20 minutes, with ethidium bromide staining and visualization under UV light.

**2.5.2 Polymerase Chain Reaction (PCR) Amplification of Genes:** The PCR master mix contained PCR buffer, MgCl₂, DMSO, DNTPs, and Taq polymerase. The reaction volume was 20µl, with 4µl master mix, 0.5µl forward and reverse primers, 1µl sample DNA, and 14µl water.

**2.5.3 PCR Conditions and Sequence Analysis:** PCR was performed using a BIO-RAD thermocycler (95°C for 30s, 30 cycles of 95°C for 30s, 54°C for 30s, 72°C for 1 min, and final elongation at 72°C for 5 min). Amplicons were separated on 1.5% agarose gel at 120V for 20 min using a 50 bp DNA ladder. Sequencing was done using the Sanger method on an ABI Prism 3130X1 Genetic Analyzer (Applied Biosystems) (Thompson *et al*., 1994)5. Sequences were aligned using ClustalW and analyzed with BLASTn (NCBI). Phylogenetic trees were constructed with MEGA11 using the Tamura-Nei model and UPGMA method (Tamura *et al*., 2021)6. Bootstrap resampling (500 replications) assessed branch support (Felsenstein, 1985)7.

**2.6 Quality Controls:** Control experiments (positive and negative) were set-up to monitor the efficiency of the media, reagents and different biochemical and serological test performed.

**2.7 Data Analysis:** Data were analyzed using SPSS Version 22. Descriptive analysis (percentages and frequencies) assessed antibiotic resistance patterns and resistance genes in Gram negative isolates. Chi-square tests, odds ratios, and multivariate logistic regression were used to identify associated risk factors, with a p-value of <0.05 considered statistically significant.

**3.0 RESULTS**

Out of 180 samples from three senatorial districts of Akwa Ibom State, 123 were positive and 57 negative. Ikot Ekpene, Uyo, and Eket had 39, 43, and 41 positive isolates, respectively. The highest prevalence was in Teaching Hospital Uyo, followed by Immanuel Hospital Eket, with the lowest in General Hospital Ikot Ekpene (Figure 2).

**Figure 2: Prevalence of Gram Negative Bacteria Isolated from under five Children in General, Teaching and Immanuel Hospital (Ikot Ekpene, Uyo and Eket)**

Characterization of Gram negative bacteria isolated from under five children in Ikot Ekpene, Uyo and Eket (Figure 3). **Immanuel Hospital, Eket** shows highest bacterial prevalence, E. coli (23.3%) was most common, followed by P. mirabilis (10.0%) and K. oxytoca (6.7%). Least common were S. marcescens and A. xylosoxidans (1.7%). K. pneumoniae, S. odorifera, P. aeruginosa, and B. vietnamiensis were absent. **Uyo Teaching Hospital** P. aeruginosa (15.0%),P. mirabilis and K. pneumoniae (13.3%) were dominant. E. fergusonii, S. odorifera, P. luteola, and B. cepacia were absent. **General Hospital, Ikot Ekpene** E. coli (13.3%) was most prevalent, followed by P. aeruginosa (10.0%). C. violaceum was absent.

**Figure 3.: Characterization of Gram Negative Bacteria Isolated from under five Children in General, Teaching and Immanuel Hospital (Ikot Ekpene, Uyo and Eket)**

The determination of Gram negative bacteria in the three hospital of study (Table 2). *E. coli* had the highest prevalence (13.9%), followed by *P. mirabilis* (10.6%) and *P. aeruginosa* (8.3%). *K. pneumoniae* (6.7%), *S. ficaria* (5.0%), *R. radiobacter* (4.4%) and *K. oxytoca* (3.9%) were also notable. The least prevalent were *B. vietnamiensis* (1.1%) and *S. odorifera* (0.6%).

**Maternal variables and sepsis risk** (Table 3).There was no significant association between sepsis and maternal age, education, or delivery place. However, children born in hospitals had a higher risk of sepsis than those born at home or in churches.

**Neonatal variables and sepsis risk** (Table 4). There was no significant correlation between sepsis and the child’s age or gender, although males had a higher risk. Higher risk groups included preterm babies (<37 weeks) with 69% positive, low birth weight infants with 71%, and normal birth weight infants with 67%. Among infants who had received antibiotics, 75% were sepsis positive. A 100% sepsis positive rate was observed among children with a previous history of sepsis, hospitalization, recent surgery, chronic medical conditions, or the use of medical equipment.

**Common neonatal signs and symptoms of sepsis** (Table 5). All infants with poor feeding, vomiting, apnea, diarrhoea, abnormal body temperature, pale skin, jaundice or seizures tested 100% positive for sepsis, while 50% of those without clinical signs still tested positive. There was no statistically significant difference in any of the clinical manifestations (*P* ˃ 0.05).

 **Table 2: Determination of Gram Negative Bacteria Isolated from under five Children in General, Teaching and Immanuel Hospital (Ikot Ekpene, Uyo and Eket).**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gram negative bacteria | Nos. of tested bacteria (n), (n/N)% in (General Hospital)  | Nos. of tested bacteria (n), (n/N)% in (Teaching Hospital) | Nos. of tested bacteria (n), (n/N)% in (Immanuel Hospital) | Total nos. of tested bacteria (n), (n/N)%  |
| *Escherichia coli* | 8 (13.3) | 3 (5.0) | 14 (23.3) | 25 (13.9) |
| *Proteus mirabilis* | 5 (8.3) | 8 (13.3) | 6 (10.0) | 19 (10.6) |
| *Pseudomonas aeruginosa* | 6 (10.0) | 9 (15.0) | 0 (0) | 15 (8.3) |
| *K. pneumoniae* | 4 (6.7) | 8 (13.3) | 0 (0) | 12 (6.7) |
| *S. ficaria* | 3 (5.0) | 3 (5.0) | 3 (5.0) | 9 (5.0) |
| *Rhizobium radiobacter* | 4 (6.7) | 1 (1.7) | 3 (5.0) | 8 (4.4) |
| *Klebsiella oxytoca* | 1 (1.7) | 2 (3.3) | 4 (6.7) | 7 (3.9) |
| *Chromobacterium violaceum* | 0 (0) | 5 (8.3) | 2 (3.3) | 7 (3.9) |
| *Serratia marcescens* | 2 (3.3) | 2 (3.3) | 1 (1.7) | 5 (2.8) |
| *Escherichia fergusonii* | 1 (1.7) | 0 (0) | 3 (5.0) | 4 (2.2) |
| *P. luteola* | 1 (1.7) | 0 (0) | 2 (3.3) | 3 (1.7) |
| *Achromobacter xylosoxidans* | 1 (1.7) | 1 (1.7) | 1 (1.7) | 3 (1.7) |
| *Burkholderia cepacia* | 1 (1.7) | 0 (0) | 2 (3.3) | 3 (1.7) |
| *B. vietnamiensis* | 1 (1.7) | 1 (1.7) | 0 (0) | 2 (1.1) |
| *S. odorifera* | 1 (1.7) | 0 (0) | 0 (0) | 1 (0.6) |
| **Total**  | **39 (65.0)** | **43 (71.7)** | **41 (68.3)** | **123 (68.3)** |

**Table 3.: Maternal Demographic Variables of the Mother in General Hospital Ikot Ekpene, Uyo Teaching Hospital Uyo and Immanuel Hospital Eket**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Maternal Demographic Variables | Total Nos. of Isolated Bacteria | Sepsis | X2 | AOR |  95% CILower Upperbound bound  |
| Positive (%) | Negative (%) |
| **Age (years)** |  |  |  |  |  |  |
| 15-24 | 40 | 26 (65) | 14 (35) | 0.156 | 1.214 | 0.464 - 3.174  |
| 25-34 | 58 | 43 (74) | 15 (26) | 0.465 | 0.749 | 0.327 - 1.718  |
| 35 and above | 82 | 54 (66) | 28 (34) | 1.117 |  |  |
| **Education** |  |  |  |  |  |  |
| FSLC | 17 | 12 (71) | 5 (29) | 0.131 | 0.714 | 0.116 - 4.409  |
| SSCE | 76 | 49 (64) | 27 (36) | 0.004 | 0.954 | 0.240 - 3.787  |
| ND | 21 | 15 (71) | 6 (29) | 0.492 | 0.563 | 0.113 - 2.801  |
| BSC/HND | 51 | 38 (75) | 13 (25) | 1.064 | 0.512 | 0.143 - 1.827  |
| MSC and above | 15 | 9 (60) | 6 (40) | 2.505 |  |  |
| **Occupation** |  |  |  |  |  |  |
| Blue collar | 113 | 77 (68) | 36 (32) | 0.040 | 0.877 | 0.243 - 3.167  |
| Whitecollar | 51 | 35 (69) | 16 (31) | 0.002 | 1.032 | 0.229 - 4.661  |
| Others | 16 | 11 (69) | 5 (31) | 0.142 |  |  |
| **Parity** |  |  |  |  |  |  |
| 1-3 | 112 | 81 (72) | 31 (28) |  |  |  |
| 4-6 | 67 | 41 (61) | 26 (39) |  |  |  |
| 7 and above | 1 | 1 (100) | 0 (0) | 2.147 |  |  |
| **Place of delivery** |  |  |  |  |  |  |
| Home | 51 | 37 (73) | 14 (27) | 0.191 | 0.811 | 0.316 - 2.077  |
| Hospital | 89 | 60 (67) | 29 (33) | 0.104 | 1.159 | 0.473 - 2.844  |
| Church | 40 | 26 (65) | 14 (35) | 0.647 |  |  |
| Others | 0 | 0 (0) | 0 (0) |  |  |  |
| **Premature rupture of membrane** |  |  |  |  |  |  |
| Yes | 39 | 26 (67) | 13 (33) | 0.002 | 1.020 | 0.458 - 2.270  |
| No | 141 | 97 (69) | 44 (31) |  |  |  |

**key:** x2 = chi-square, aor = adjusted odd ratio, ci = confidence interval, fslc = first school leaving certificate, ssce = senior secondary certificate examination, nd = national diploma, hnd = higher national diploma, bsc = bachelor of science, msc = master of science.

**Table 4: Neonatal Demographic Variables of the Children in General Hospital Ikot Ekpene, Uyo Teaching Hospital Uyo and Immanuel Hospital Eket**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Neonatal Demographic Variables  | Total Nos. of Isolated Bacteria | Sepsis | X2 | AOR |  95% CILower Upperbound bound  |
| Positive (%) | Negative (%) |
| **Age (years)** |  |  |  |  |  |  |
| 0-1 | 30 | 22 (73) | 8 (27) | 0.841 | 2.496 | 0.353 17.634 |
| 1.1-2 | 33 | 22 (67) | 11 (33) | 0.057 | 1.247 | 0.202 7.704 |
| 2.1-3 | 45 | 32 (71) | 13 (29) | 0.048 | 0.833 | 0.162 4.285 |
| 3.1-4 | 32 | 20 (63) | 12 (38) | 0.426 | 1.759 | 0.322 9.596 |
| 4.1-5 | 40 | 27 (68) | 13 (33) | 1.764 |  |  |
| **Sex** |  |  |  |  |  |  |
| Male | 87 | 60 (69) | 27 (31) | 1.465 | 1.980 | 0.655 5.984 |
| Female | 93 | 63 (68) | 30 (32) |  |  |  |
| **Preterm (< 37 weeks)** |  |  |  |  |  |  |
| Yes | 45 | 31 (69) | 14 (31) |  |  |  |
| No | 135 | 92 (68) | 43 (32) |  |  |  |
| **Fullterm (> 37 weeks)** |  |  |  |  |  |  |
| Yes | 134 | 91 (68) | 43 (32) |  |  |  |
| No | 46 | 32 (70) | 14 (30) |  |  |  |
| **Birthweight (BW) (kg)** |  |  |  |  |  |  |
| Low BW (<2.5) | 51 | 36 (71) | 15 (29) |  |  |  |
| Normal BW (>2.5) | 128 | 86 (67) | 42 (33) |  |  |  |
| High BW (above 3.5) | 1 | 1 (100) | 0 (0) |  |  |  |
| **Antibiotic use** |  |  |  |  |  |  |
| Yes | 56 | 42 (75) | 14 (25) |  |  |  |
| No | 124 | 81 (65) | 43 (35) |  |  |  |
| **Previous history of septicaemia** |  |  |  |  |  |  |
| Yes | 32 | 32 (100) | 0 (0) |  |  |  |
| No | 148 | 91 (61) | 57 (39) |  |  |  |
| **Hospitalized children** |  |  |  |  |  |  |
| Yes | 61 | 61 (100) | 0 (0) |  |  |  |
| No | 119 | 62 (52) | 57 (48) |  |  |  |
| **Recent surgery** |  |  |  |  |  |  |
| Yes | 2 | 2 (100) | 0 (0) |  |  |  |
| No | 178 | 121 (68) | 57 (32) |  |  |  |
| **Chronic medical condition** |  |  |  |  |  |  |
| Yes | 36 | 36 (100) | 0 (0) |  |  |  |
| No | 144 | 87 (60) | 57 (40) |  |  |  |
| **Child with medical equipment** |  |  |  |  |  |  |
| Yes | 27 | 27 (100) | 0 (0) |  |  |  |
| No | 153 | 96 (63) | 57 (37) |  |  |  |

**key:** x2 = chi-square, aor = adjusted odd ratio, ci = confidence interval, bw = birth weight

|  |  |  |
| --- | --- | --- |
| Neonatal Signs and symptoms | Total Nos. of Isolated Bacteria | Sepsis |
|  Positive (%) |  Negative (%) |
| **Not eating/feeding well** |  |  |  |
| Yes | 54 | 54 (100) | 0 (0) |
| No | 126 | 69 (55) | 57 (45) |
| **Low body temperature****(< 36°c)** |  |  |  |
| Yes | 35 | 35 (100) | 0 (0) |
| No | 145 | 88 (61) | 57 (39) |
| **High body temperature****(> 37°c)** |  |  |  |
| Yes | 16 | 16 (100) | 0 (0) |
| No | 164 | 107 (65) | 57 (35) |
| **Cool extremities** |  |  |  |
| Yes | 30 | 30 (100) | 0 (0) |
| No | 150 | 93 (62) | 57 (38) |
| **Jaundice** |  |  |  |
| Yes | 5 | 5 (100) | 0 (0) |
| No | 175 | 118(67) | 57 (33) |
| **Apnea** |  |  |  |
| Yes | 42 | 42 (100) | 0 (0) |
| No | 138 | 81 (59) | 57 (41) |
| **Pale skin colour** |  |  |  |
| Yes | 14 | 14 (100) | 0 (0) |
| No | 166 |  109 (66) | 57 (34) |
| **Seizure** |  |  |  |
| Yes | 3 | 3 (100) | 0 (0) |
| No | 177 | 120 (68) | 57 (32) |
| **Abdominal swelling** |  |  |  |
| Yes | 18 | 18 (100) | 0 (0) |
| No | 162 | 105 (65) | 57 (35) |
| **Vomiting** |  |  |  |
| Yes | 47 | 47 (100) | 0 (0) |
| No | 133 | 76 (57) | 57 (43) |
| **Diarrhoea** |  |  |  |
| Yes | 41 | 41 (100) | 0 (0) |
| No | 139 | 82 (59) | 57 (41) |
| **Clinical signs and symptoms** |  |  |  |
| Positive | 65 | 65 (100) | 0 (0) |
| Negative | 115 | 58(50) | 57 (50) |

**Table 5: Neonatal Signs and Symptoms of the Children in General Hospital Ikot Ekpene, Uyo Teaching Hospital Uyo and Immanuel Hospital Eket**

### **Antibiotic susceptibility of Gram negative bacteria**. This study observed that Gram negative bacteria had the highest susceptibility to tetracycline (TET), gentamicin (GEN), chloramphenicol (CHL), ciprofloxacin (CIP), amikacin (AMK), and vancomycin (VAN). Resistance was most prominent against tetracycline, cotrimoxazole, cefotaxime (CTX), cefuroxime (CRX), ceftriaxone (CTR), ceftazidime (CPZ), ciprofloxacin, and meropenem (MEM). Key findings include; ***Escherichia coli*** and ***Proteus mirabilis*** were 100% susceptible to AMK, with *E. coli* resistant to CTX and CPZ and *P. mirabilis* resistant to CPZ. ***Pseudomonas aeruginosa*** showed 40% susceptible to vancomycin and 100% resistance to cephalosporins and MEM. ***Klebsiella pneumoniae*** was 100% susceptible to AMK and resistant to CTX, CPZ and MEM. ***Serratia ficaria*** was 100% susceptible to AMK, TET and VAN and resistance to CTX and MEM (Table 6).

**Table 6: Percentage Antibiotic Susceptibility of Gram Negative Bacteria (*E. coli*, *P. mirabilis*, *P. aeruginosa*, *K. pneumonia*, *S. ficaria*) Isolated from under five Children in General, Teaching and Immanuel Hospital, (Ikot Ekpene, Uyo and Eket).**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Nos. of isolated Gram negative bacteria  | AP | TET (%) | COT (%) | GEN (%) | CRX (%) | CHL (%) | CTR (%) | CTX (%) | CIP (%) | AMK (%) | VAN (%) | CPZ (%) | MEM (%) |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *E. coli* (25) | S | 13(52.0) | 10(40.0) | 22(88.0) | 2(8.0) | 21(84.0) | 2(8.0) | 0 | 11(44.0) | 25(100.0) | 11(44.0) | 0 | 0 |
|  | R | 10 (40.0) | 13 (52.0) | 3 (12.0) | 19(76.0) | 0 | 13 (52.0) | 25(100.0) | 10 (40.0) | 0 | 12 (48.0) | 25 (100.0) | 24 (96.0) |
| *P. mirabilis*(19) | S | 12 (63.2) | 11 (57.9) | 16 (84.2) | 1 (5.3) | 5 (26.3) | 3 (15.8) | 0 | 7 (36.8) | 19 (100.0) | 14 (73.7) | 0 | 1 (5.3) |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | R | 5 (26.3) | 8 (42.1) | 2 (10.5) | 15 (78.9) | 6 (31.6) | 13 (68.4) | 18 (94.7) | 4 (21.1) | 0 | 3 (15.8) | 19(100.0) | 18 (94.7) |
| *P. aeruginosa* (15) | S | 1(6.7) | 0 | 5 (33.3) | 0 | 2 (13.3) | 0 | 0 | 5(33.3) | 5 (33.3) | 6 (40.0) | 0 | 0 |
|  | R | 13 (86.7) | 13 (86.7) | 10 (66.7) | 15 (100.0) | 13 (86.7) | 15 (100.0) | 15 (100.0) | 10 (66.7) | 10 (66.7) | 9 (60.0) | 15 (100.0) | 15 (100.0) |
| *K. pneumonia* (12) | S | 2(16.7) | 4(33.3) | 10(83.3) | 0 | 11(91.7) | 0 | 0 | 1(8.3) | 12(100.0) | 8(66.7) | 0 | 0 |
|  | R | 10(83.3) | 8(66.7) | 1(8.3) | 11(91.7) | 0 | 11(91.7) | 12(100.0) | 11(91.7) | 0 | 4(33.3) | 12(100.0) | 12(100.0) |
| *S. ficaria* (9) | S | 9(100.0) | 8(88.9) | 8(88.9) | 4(44.4) | 6(66.7) | 0 | 0 | 8(88.9) | 9(100.0) | 9(100.0) | 1(11.1) | 0 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | R | 0 | 0 | 1(11.1) | 3(33.3) | 2(22.2) | 8(88.9) | 9(100.0) | 1(11.1) | 0 | 0 | 7(77.8) | 9(100.0) |

**key:** tet 10µg, cot 25µg, gen 10µg, amk 30µg, van 30µg, crx 30µg, ctr 30µg, ctx 30µg, cpz 30µg, chl 10µg, cip 5µg, mem 10µg. ap - antibiotic pattern, s - susceptibility, r - resistance.

The determination of antibiotic resistance pattern of Gram negative isolates (*E*. *coli*, *P*. *mirabilis, P*. *aeruginosa*) obtained from sepsis in under five children (Table 7).The study found that all Gram negative isolates exhibited resistance to cephalosporins and carbapenems. Among 25 *E. coli* isolates, 30-75% showed resistance to antibiotics such as CRX, CTR, CTX, TET, CIP, COT, VAN, GEN and MEM. In *P. mirabilis* (19 isolates), 30-70% were resistant to CRX, CTR, CTX, CPZ, CIP, COT, CHL and MEM. *P. aeruginosa* (15 isolates) displayed 40-100% resistance to antibiotics like CRX, CTR, CTX, CPZ, CIP, TET, COT, CHL, VAN, GEN, AMK and MEM. *K. pneumoniae* (12 isolates) showed 40-80% resistance to CRX, CTR, CTX, CPZ, CIP, COT, TET, VAN, AMK and MEM, including *S. ficaria* and *R. radiobacter* (Table.7).The findings emphasize the need for better antimicrobial stewardship in managing pediatric sepsis cases.

**Table 7: Determination of Antibiotic Resistance Pattern of Gram Negative Isolates (*E*. *coli*, *P*. *mirabilis, P*. *aeruginosa*) obtained from Sepsis in under five Children in General, Teaching and Immanuel Hospital (Ikot Ekpene, Uyo and Eket)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Gram negative bacteria | Nos.of isolated Gram negative bacteria | Nos. of resistance antibiotic (X) | MARI = X/Y (%) | Antibiotic resistance pattern (ARP) | Antibiotic resistance class (ARC) |
|  |  |  |  |  |  |
| *Escherichia coli* | 2 | 9 | 9/12 = 0.75 = 75  | CRX, CTR, CTX, TET, CIP, COT, VAN, GEN, MEM | Ceph, tetra, fluoro, sulf, glyco, aminogly, carb |
|  | 2 | 8 | 8/12 = 0.7 = 70 | CRX, CTX, CPZ, TET, COT, CIP, AMK, MEM | Ceph, sulf, tetra, fluoro, aminogly, carb |
|  | 6 | 7 | 7/12 = 0.6 = 60 | CRX, CTR, CTX, CPZ, COT, CIP, MEM | Ceph, sulf, fluoro, carb |
|  | 5 | 6 | 6/12 = 0.5 = 50 | CRX, CTX, CPZ, TET, VAN, MEM | Ceph, tetra, glyco, carb |
|  | 8 | 5 | 5/12 = 0.4 = 40 | CRX, CTR, CTX, CPZ, MEM | Ceph, carb |
|  | 2 | 4 | 4/12 = 0.3 = 30 | CTR, CTX, CPZ, MEM | Ceph, carb |
|  | **25** |  |  |  |  |
| *Proteus mirabilis* | 2 | 8 | 8/12 = 0.7 = 70 | CRX, CTR, CTX, CPZ, CIP, COT, CHL, MEM  | Ceph, fluoro, sulf, chlo, carb |
|  | 11 | 6 | 6/12 = 0.5 = 50 | CTX, CPZ, TET, COT, VAN, MEM | Ceph, tetra, sulf, glyco, carb |
|  | 5 | 5 | 5/12 = 0.4 = 40 | CRX, CTX, CPZ,CHL, MEM | Ceph, chlo, carb |
|  | 1 | 4 | 4/12 = 0.3 = 30 | CRX, CTX, CPZ, MEM | Ceph, carb |
|  | **19** |  |  |  |  |
| *Pseudomonas aeruginosa* | 9 | 12 | 12/12 = 1 = 100 | CRX, CTR, CTX, CPZ, CIP, TET, COT, CHL, VAN, GEN, AMK, MEM | Ceph, fluoro, tetra, sulf, chlo, glyco, aminogly, carb  |
|  | 1 | 11 | 11/12 = 0.9 = 90 | CRX, CTR, CTX, CPZ, CIP, TET, COT, CHL, GEN, AMK, MEM | Ceph, fluoro, tetra, sulf, chlo, aminogly, carb  |
|  | 2 | 8 | 8/12 = 0.7 = 70 | CRX, CTR, CTX, CPZ, TET, COT, CHL, MEM | Ceph, tetra, sulf, chlo, carb  |
|  | 1 | 7 | 7/12 = 0.6 = 60 | CRX, CTR, CTX, CPZ, TET, COT, MEM | Ceph, tetra, sulf, carb  |
|  | 1 | 6 | 6/12 = 0.5 = 50 | CRX, CTR, CTX, CPZ, CHL, MEM | Ceph, chlo, carb |
|  | 1 | 5 | 5/12 = 0.4 = 40 | CRX, CTR, CTX, CPZ, MEM | Ceph, carb |
|  | **15** |  |  |  |  |

**Table 8: Determination of Antibiotic Resistance Pattern of Gram Negative Isolates (*K*. *pneumoniae*, *S*. *ficaria*, *R*. *radiobacter*) obtained from Sepsis in under five Children in General, Teaching and Immanuel Hospital (Ikot Ekpene, Uyo and Eket)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Gram negative bacteria | Nos.of isolated Gram negative bacteria | Nos. of resistance antibiotic (X) | MARI = X/Y (%) | Antibiotic resistance pattern (ARP) | Antibiotic resistance class (ARC) |
| *Klebsiella pneumoniae* | 1 | 10 | 10/12 = 0.8 = 80  | CRX, CTR, CTX, CPZ, CIP, COT, TET,VAN, AMK, MEM | Ceph, fluoro, sulf, tetra, glyco, aminogly, carb |
|  | 4 | 9 | 9/12 = 0.75 = 75 | CRX, CTR, CTX, CPZ, TET, COT, CIP, VAN, MEM | Ceph, tetra, sulf, fluoro, glyco, carb |
|  | 1 | 8 | 8/12 = 0.7 = 70 | CRX, CTR, CTX, CPZ, TET, COT, CIP, MEM | Ceph, tetra, sulf, fluoro, carb |
|  | 4 | 7 | 7/12 = 0.6 = 60 | CTR, CTX, CPZ, TET, COT, CIP, MEM | Ceph, tetra, sulf, fluoro, carb |
|  | 1 | 6 | 6/12 = 0.5 = 50 | CRX, CTR, CTX, CPZ, CIP, MEM | Ceph, fluoro, carb |
|  | 1 | 5 | 5/12 = 0.4 = 40 | CRX, CTR, CTX, CPZ, MEM | Ceph, carb |
|  | **12** |  |  |  |  |
| *Serratia ficaria* | 2 | 6 | 6/12 = 0.5 = 50 | CTR, CTX, CPZ, CIP, GEN, MEM  | Ceph, fluoro, aminogly, carb |
|  | 2 | 5 | 5/12 = 0.4 = 40 | CTR, CTX, CPZ, CHL, MEM | Ceph, chloro, carb |
|  | 4 | 4 | 4/12 = 0.3 = 30 | CTR, CTX, CPZ, MEM | Ceph, carb |
|  | 1 | 2 | 2/12 = 0.2 = 20 | CTX, MEM | Ceph, carb |
|  | **9** |  |  |  |  |
| *Rhizobium radiobacter* | 2 | 7 | 7/12 = 0.6 = 60 | CRX, CTR, CTX, CPZ, TET, COT, MEM | Ceph, tetra, sulf, carb  |
|  | 3 | 6 | 6/12 = 0.5 = 50 | CRX, CTR, CTX, CPZ, COT, MEM | Ceph, sulf, carb  |
|  | 1 | 5 | 5/12 = 0.4 = 40 | CRX, CTR, CTX, CPZ, MEM | Ceph, carb  |
|  | 2 | 3 | 3/12 = 0.3 = 30 | CTR, CTX, CHL | Ceph, chloro  |
|  | **8** |  |  |  |  |

**key:** tet 10µg – tet or broad spectrum, cot 25µg - sulf, gen 10µg, amk 30µg aminogly, van 30µg - glyco, crx 30µg, ctr 30µg, ctx 30µg cpz 30µg - ceph, chl 10µg - chl, cip 5µg - fluoroquinolones (quinolones) (fluoro), mem 10µg - carb, mari - multiple antibiotic resistance index mari = x/y where x = number of antibiotics to which test include displayed resistance, y = the total number of antibiotics to which the test organism has been evaluated for sensitivity.

DNA obtain from *E. coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Figure 4). The amplification was very high in *Pseudomonas aeruginosa* compare to *E. coli* and *Klebsiella pneumoniae*.

 1 2 3

**Figure 4:** **DNA electrophoresis Image of *E. coli*, *P. aeruginosa* and *K. pneumoniae***

1 = *E. coli*; 2 = *P. aeruginosa*; 3 = *K. pneumoniae*

FimH (type 1fimbrae) and CNF1 (cytotoxic necrotizing factor 1) gene (Figure 5). FimH gene amplified only *Klebsiella* and absence in *E. coli* and *Pseudomonas* whereas CNF1 gene amplified *E. coli, Pseudomonas* and *Klebsiella* at a very low base pair which may be related to primers dimers.

**400bp**

 M 1 2 3 4 5 6

**Figure 5:** **Electrophoresis Image of FimH gene and CNF1 gene**

M = DNA ladder, 1 = fimh gene for *E. coli*; 2 = fimh gene for *P. aeruginosa*; 3 = fimh gene for *K. pneumoniae*,4 = cnf1 gene for *E. coli*; 5 = cnf1 gene for *P. aeruginosa*; 6 = cnf1 gene for *K. pneumoniae.*

PAGS and PASS gene, in PAGS gene the primer amplify the genes of *E. coli, Pseudomonas* and *Klebsiella* (Figure 6). Although the genes amplified *E. coli, Pseudomonas* and *Klebsiella*. *Pseudomonas* sequencing has high quality with fine peak followed by *Klebsiella* and *E. coli*. PASS also amplify the genes of *E. coli*, *Pseudomonas* and *Klebsiella* with high quality fine peak of *Pseudomonas* than others.

The Hlyc, SHV, TEM, and CTX-M genes were amplified at 50-60°C (Figure 7). Hlyc and SHV genes were detected in the three bacteria but had low base pairs. In TEM gene there was amplification for *Pseudomonas* and *Klebsiella* and was absent in *E. coli* indicating that the gene region that was used to design the primer was not presence in the organism. CTX-M genes were absent in all three bacteria, suggesting no expression of the resistance gene.

**400bp**

 M 1 2 3 4 5 6

**Figure 6:** **Electrophoresis Image of PAGS and PASS gene for *E. coli*, *P. aeruginosa* and *K*. *pneumoniae***

M = DNA ladder, 1 = pags gene for *E. coli*; 2 = pags gene for *P. aeruginosa*; 3 = pags gene for *K.**pneumoniae*,4 = pass gene for *E. coli*; 5 = pass gene for *P. aeruginosa*; 6 = pass gene for *K. pneumoniae*

**400bp**

 M 1 2 3 4 5 6 7 8 9 10 11 12

**Figure 7:** **Electrophoresis Image of Hlyc, SHV, TEM and CTX-M genes for *E. coli*, *P. aeruginosa* and *K*. *pneumoniae***

 M = DNA ladder, 1= hlyc gene for *E. coli*; 2 = hlyc gene for *P. aeruginosa*; 3 = hlyc gene for *K. pneumoniae*; 4 = shv gene for *E. coli*; 5 = shv gene for *P. aeruginosa*; 6 = shv gene for *K. pneumoniae*; 7 = tem gene for *E. coli*; 8 = tem gene for *P. aeruginosa*; 9 = tem gene for *K. pneumoniae*; 10 = ctx-m gene for *E. coli*; 11= ctx-m gene for *P. aeruginosa*; 12 = ctx-m gene for *K. pneumoniae.*

The *Klebsiella* FimH gene phylogeny (Figure 8). showed two major clusters that share a common ancestor, one containing several subclusters of *Klebsiella* FimH genes, and the other containing *E. coli* strain T43 chromosomes, members of each are more closely related than they are with members of the other cluster. The *E. coli, Pseudomonas* and *Klebsiella* PAGS gene tree (Figure 9) had three clusters, with *Klebsiella* and *Pseudomonas* being very closely related with several subclusters which share a most recent common ancestor, while *E. coli* also share a common ancestor but not closely related to others. In the PASS gene phylogeny (Figure 10), two clusters were observed, with *E. coli* and *Pseudomonas* being most closely related than *Klebsiella*.

****

**Figure 8: The Phylogenetic Relationship of *Klebsiella* FimH Gene**

This analysis involved 18 nucleotide sequences, codon positions included were 1st+2nd+3rd+noncoding, all ambiguous positions were removed for each sequence pair (pairwise deletion option), and there were a total of 443 positions in the final dataset.

**Figure 9: The Phylogenetic Relationship of *E. coli*, *Pseudomonas* and *Klebsiella* PAGS Gene**

This analysis involved 21 nucleotide sequences, codon positions included were 1st+2nd+3rd+noncoding, all ambiguous positions were removed for each sequence pair (pairwise deletion option), there were a total of 949 positions in the final dataset, evolutionary analyses were conducted in mega11 (Tamura *et al*., 2021)6.



**Figure 10: The phylogenetic relationship of *E. coli*, *Pseudomonas* and *Klebsiella* PASS gene.**

This analysis involved 12 nucleotide sequences, codon positions included were 1st+2nd+3rd+noncoding, all ambiguous positions were removed for each sequence pair (pairwise deletion option), there were a total of 940 positions in the final dataset.

The phylogenetic relationship of *Pseudomonas* and *Klebsiella* TEM gene (Figure 11). The phylogenetic tree has two major clusters that share a common ancestor, cluster one is made up of isolate 2 TEM gene (*Pseudomonas*) and isolate 3 TEM gene (*Klebsiella*) which share a most recent common ancestor than cluster two, members of each other are more closely related than they are with members of the other cluster. However, *Pseudomonas* and *Klebsiella* share a common ancestor than *E. coli.*

****

**Figure 11: The phylogenetic relationship of *Pseudomonas* and *Klebsiella* TEM gene.**

This analysis involved 14 nucleotide sequences, codon positions included were 1st+2nd+3rd+noncoding, all ambiguous positions were removed for each sequence pair (pairwise deletion option), there were a total of 926 positions in the final dataset.

**4.0 DISCUSSION**

This study found a 68.3% prevalence of Gram negative bacteria, aligning with previous Nigerian studies (Miranda *et al*., 2024; Godfrey *et al*., 2022)8,9. Factors such as antibiotic resistance, environmental conditions and lifestyle changes contribute to this high burden. Contrary to this work, Aletayeb *et al*., (2011)10 in Nigeria reported a lower prevalence (4.1%) due to maternal/neonatal antibiotic use, blood culture techniques, and misdiagnosis (Bansal *et al*., 2004; Agnihotri *et al*., 2004; Raha *et al*., 2014)11,12,13. Differences in bacterial isolates may be attributed to hygiene, antibiotic exposure, diagnostic approaches and geographical variation (Giannoni *et al*., 2018)14.

*E. coli* (13.9%) was the most frequent isolate, consistent with prior Nigerian studies (Peterside *et al*., 2015; Godfrey *et al*., 2022)15,9. The absence of *K. pneumoniae* and *P. aeruginosa* in one hospital may reflect differences in clinical sampling or regional variation. Maternal factors also influenced sepsis risk, with PROM increasing susceptibility, while mothers with SSCE had lower exposure risks. Sepsis was more common in male infants under one year, consistent with past studies (Rudd *et al*., 2020; Godfrey *et al*., 2022)2,9. Antibiotic use was strongly associated with sepsis (OR=4.959), supporting concerns about antibiotic overuse and resistance.

Multidrug resistance was a major concern, with *Escherichia spp*., *P. mirabilis*, and *K. pneumoniae* showing 100% susceptibility to amikacin, suggesting its efficacy. However, resistance to cephalosporins (CTX, CPZ, MEM) was widespread, consistent with previous findings (Bai *et al*., 2021; Ahmad *et al*., 2021)16,17. The detection of FimH, PAGS, PASS and blaTEM, genes underscores the growing challenge of antibiotic resistance. This study highlights the urgent need for antibiotic stewardship and improved infection control measures, emphasizing geographical variations in resistance patterns to guide effective treatment strategies.

**5.0 CONCLUSIONS**

This study highlights the high prevalence of antibiotic resistance among Gram negative organisms causing sepsis in under-five children, with cefotaxime showing significant resistance. While antibiotics remain crucial in infection control, their misuse accelerates resistance. Pediatricians must regulate antibiotic use and consider timely replacements to enhance efficacy. Notably, amikacin demonstrated 100% susceptibility, indicating its potential as a preferred treatment option. *E. coli*, *P. mirabilis*, *P. aeruginosa*, *K. pneumoniae*, *S. ficaria* and *R.* *radiobacter* remain the predominant pathogens. Gene relatedness analysis revealed a link between *K. pneumoniae* and *P. aeruginosa*. Early identification through screening tools and sepsis bundles in emergency departments is essential for timely intervention and improved outcomes.

**CONSENT**

All authors declare that ‘written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal."

**ETHICAL APPROVAL**

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

**REFERENCES**

1. Basco, W. T. Jr. (2021). The febrile infant: New AAP guidance for the first 2 months of life. Medscape News and Perspective, 19, 7. <https://www.medscape.com/viewarticle/954462>.

2. Rudd, K. E., Johnson, S. C., Agesa, K. M., Shackelford, K. A., Tsoi, D., & Kievlan, D. R., et al. (2020). Global, regional and national sepsis incidence and mortality: Analysis for the global burden of disease study. *Lancet,* 395 (10219), 200-211.

3. Olorukooba, A. A., Ifusemu, W. R., Ibrahim, M. S., Jibril, M. B., Amadu, L., & Lawal, B. B. et al. (2020). Prevalence and factors associated with neonatal sepsis in a tertiary hospital, north west Nigeria. *Nigerian Medical Journal,* 61(2), 60-66.

4. National Population Commission of Nigeria. (2011). The population projection: Map showing the three senatorial districts in Akwa Ibom State. <https://www.nationalpopulation.gov.ng>

5. Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22 (22), 4673-4680.

6. Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA 11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution. <https://doi.org/10.1093/molbev/msab120>.

7. Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39, 783-791.

8. Miranda, S., Harahap, A., Husada, D., & Faramarisa, F. N. (2024). Microbial pattern of neonatal sepsis in the neonatal intensive care unit of Dr. Ramelan navy central hospital. I*nternational Journal of Pediatrics,* 2024, 1-8.

9. Godfrey, E., Majaliwa, E., & Assenga, E. N. (2022). Aetiology, antimicrobial susceptibility and outcome of children with sepsis, admitted at Muhimbili national hospital, Dares Salaam. *Pan African Medical Journal*, 42 (167), 1937-8688.

10. Aletayeb, S. M. H., Khosravi, A. D., Dehdashtian, M., Kompani, F., Mortazavi, S. M., & Aramesh, M. R. (2011). Identification of bacterial agents and antimicrobial susceptibility of neonatal sepsis: A 54-month study in a tertiary hospital. *African Journal of Microbiology Research*, 5 (5), 528-531.

11. Bansal, S., Jain, A., Agarwal, J., & Malik, G. K. (2004). Significance of coagulase negative staphylococci in neonates with late onset septicaemia. *Indian Journal of Pathology and Microbiology*, 47 (4), 586-568.

12. Agnihotri, N., Kaistha, N., & Gupta, V. (2004). Antimicrobial susceptibility of isolates from neonatal septicemia. *Japanese Journal of Infectious Disease*, 57 (6), 273-275.

13. Raha, B. K., Baki, M. A., Begum, T., Nahar, N., Jahan, N., & Begum, M. (2014). Clinical, bacteriological profile and outcome of neonatal sepsis in a tertiary care hospital. *Medicine Today,* 26 (1), 18-21*.*

14. Giannoni, E., Agyeman, P. K. A., Stocker, M., Posfay-Barbe, K. M., Heininger, U., & Spycher, B. D., et al. (2018). Neonatal sepsis of early onset and hospital-acquired and community-acquired late onset*:* A prospective population-based cohort study*. The Journal of Pediatrics,* 201, 106-114.

15. Peterside, O., Pondei, K., & Akinbami, F. O. (2015). Bacteriological profile and antibiotic susceptibility pattern of neonatal sepsis at a teaching hospital in Bayelsa State. *Nigeria Tropical Medicine Health*, 43 (3), 183-190.

16. Bai, X., Wei, Q., Duan, T., Yi, Y., Peng, H., & Hu, L. (2021). Predominance of Gram negative infections a cause of neonatal sepsis among low birth weight preterm infants. *Journal of Laboratory Medicine*, 45 (1), 7-12.

17. Ahmad, A., Sarwar, N., Aslam, R., Ali, S., Aslam, B., & Arshad, M. A., et al. (2021). Pattern of clinical drug resistance and occurrence of Gram negative bacterial neonatal sepsis at a tertiary care hospital. *Pakistan Journal of Pharmaceutical Sciences*, 34 (5), 1873-1878.