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Phenotypic Characterization and Antibiogram Study of *Escherichia coli* and *Klebsiella Pneumoniae* among Neonates in Mile - Four Hospital, South Eastern, Nigeria

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Abstract

Background: Gram-negative bacteria are a primary source of illness in newborns. **Objective:** The purpose of this study was to isolate, characterise, and analyse the antibiotic resistance patterns of some Gram-negative bacteria that are involved in illnesses in newborns in Ebonyi State.

Methods: A total of 200 unique blood and stool samples were gathered from infants in the Nursery Unit of Mile Four Hospital Abakaliki. Demographic information was taken from the patients' medical records. The samples were promptly sent to the Applied Microbiology Laboratory Complex at Ebonyi State University Abakaliki for bacteriological testing. The examination followed conventional microbiological protocols for isolating and identifying bacteria. The isolates underwent antibiotic susceptibility testing using the Kirby – Bauer disc diffusion method. The results were interpreted using the Clinical Laboratory Standard Institute (CLSI) zone diameter breakpoints, and the multiple antibiotic resistance index (MARI) was established.

Findings: The findings indicated that there was a total isolation rate of 17.5%, with *E. coli* and *K. pneumoniae* isolates accounting for 9.5% and 8% respectively. The demographic data of patients indicated that the isolation rate was 54.5% for male patients and 45.5% for female patients. The *K. pneumoniae* and *E. coli* isolates showed a high level of resistance, ranging from 50% to 100%, against amikacin, ertapenem, meropenem, ceftazidime, ceftriaxone, cefepime, sulphamethoxazole, and novobiocin. They also exhibited multi-drug resistance (MDR) with a minimum inhibitory concentration (MIC) value of ≥0.5. However, they were completely susceptible to gentamicin, ofloxacin, and ciprofloxacin.

Conclusion: This analysis supports the need of regularly washing hands and using gloves among healthcare personnel and hospital residents. Thoroughly examining pregnant mothers and newborns is crucial in order to minimise the potential hazards linked to probable exposure to *K. pneumoniae* and *E. coli.*

INTRODUCTION

Neonatal infections are medical conditions that affect newborns and are acquired either during prenatal development or within the first few weeks of life, which is known as the neonatal period. These infections can be acquired through vertical transmission from mother to child, during childbirth in the birth canal, or after birth[1]. Certain prenatal infections, such as HIV, hepatitis B, and malaria, may not manifest symptoms until a later stage. Preterm or low birth weight neonates are at an elevated risk of infection. Infant respiratory distress syndrome is frequently observed in premature newborns and can lead to enduring adverse effects. Additionally, it can also occur as a result of an infection. Neonatal respiratory tract illnesses can sometimes make individuals more prone to future respiratory infections and inflammatory reactions associated with lung disease[2] .

The Gram-negative bacteria commonly associated with infections in neonates include *Klebsiella pneumoniae, Serratia marcescens, Pseudomonas aeruginosa, Chlamydiatrachomatis, Escherichia coli, and Enterobacter cloacae*[3].Neonatal infections can be effectively treated with antibiotics, particularly when the pathogen is promptly diagnosed. Advancements in technology have significantly enhanced pathogen identification, reducing the need on culture techniques. However, despite these improvements, the reduction in neonatal mortality has not progressed at the same rate and stays between 20% to 50%

[4].Although preterm neonates are especially susceptible, all neonates are susceptible to infection. Neonatal infection can also be linked to preterm rupture of membranes, which significantly raises the likelihood of neonatal sepsis by creating a pathway for bacteria to enter the womb before the baby is born [5.] Neonatal infection can cause significant distress to the family and prompts professionals to take immediate action in treatment. Ongoing research aims to enhance the treatment of infections and implement preventive measures in mothers to prevent infections in infants. newborn infections in industrialised nations are often treated in the newborn intensive care unit (NICU). The maternal gastrointestinal and genitourinary tract is frequently identified as the source of pathogenic bacteria and some other diseases. A significant number of maternal infections caused by these organisms do not exhibit any symptoms in the mother. Additional maternal diseases that can be passed on to the baby during pregnancy or childbirth include bacterial and viral sexually transmitted infections [6]. The infant's capacity to withstand infection is constrained by its underdeveloped immune system. Furthermore, the neonate's immune system may exhibit responses that can give rise to complications in treatment, such as the secretion of inflammatory substances. Infants with congenital abnormalities of the immune system also experience impaired abilities to combat these illnesses [7.]

However, since there is scanty data from the current study area, the study hence was conducted with the purpose to isolate, identify and investigate the susceptibility of the bacteria involved in illnesses in newborns at Mile- four Hospital, Abakaliki to different antibiotics in order to ensure effective treatment.

MATERIALS AND METHODS

Study Area

The research was carried out at Abakaliki, located in Ebonyi State, Nigeria. Ebonyi State is situated in the southeastern region of Nigeria, between longitude 7.30' and 8.30'E and latitude 5.40' and 6.45'N. The State was established on October 1, 1996 by merging the old Abia and Enugu States, with Abakaliki serving as its capital. It is bordered to the north by Benue State, to the west by Enugu State, to the east by Cross River State, and to the south by Abia State. The State consists of thirteen Local Government Areas (LGAs), specifically Abakaliki, Ebonyi, Ishielu, Ohaukwu, lzzi, lkwo, Ezza North, Ezza South, Afikpo North, Afikpo South, lvo, Ohaozara, and Onicha LGAs. Abakaliki is home to numerous governmental and privately-
owned health centres. These facilities offer laboratory services for prenatal screening.

Ethical considerations

The researchers obtained and received ethical permission from the Ethics Committee of the participating hospitals. Prior to the start of the study, consent was acquired from the Administrators of the Hospitals. All prenatal patients included in the study willingly provided written consent after receiving a comprehensive explanation of the study's purpose.

Study Population and Design

Clinical samples (blood and stool) were collected from both male and female newborns from 0 – 28 days of age comprising of out-born and new-born patients in Nursery Unit Mile Four Hospital Abakaliki. Patients placed in the firefly machine were included in the study while those (still-birth) placed inside the resusitae machine, incubator and emergency surgery cases were excluded in this study.

Collection and preparation of samples

Blood and stool samples were collected from the newborns by medical personnel in the Nursery Unit of the hospital using sterile cotton swabs and needles. A total of 200 non – repeatable clinical samples (blood and stool) were randomly collected from patients including those with cases of jaundice were included in the sample collection. Demographic data (sex and age) of patients were all noted. The collected samples were immediately transported in an

icepack to Microbiology Laboratory Unit of Ebonyi State University Abakaliki, for bacteriological analysis.

Processing of Clinical Specimens

Aseptically, all collected stool and blood samples were suspended separately in a sterilized Brainheart infusion broth (Oxoid UK), and incubated at 37 ^{o}C for 24 h. The suspended swab and blood samples from the turbid bacterial growth was sub cultured onto Eosin methylene blue agar and MacConkey agar (OxoidUk) medium, and incubated for 24 h. *Escherichia coli* and *Klebsiella pneumoniae* colonies were identified based on its colony characteristics on MacConkey agar. The colonies were aseptically examined macroscopically and microscopically for their cellular characteristics and cultural morphology. The isolates were characterized based on their colonial morphology (color and texture), microscopic techniques (Gram staining) and biochemical characteristics which include indole, oxidase, citrate and catalase tests.

Antibiotic Susceptibility Testing

Antibiotic susceptibility test of the isolates were carried out using Kirby-Bauer disc diffusion Method according to Clinical and Laboratory Standards Institute [8]. A suspension was made from a 24 h growth of the test organism in sterile water to match 0.5 McFarland turbidity standard. This was seeded on the entire surface of solidified Muller – Hinton agar (Thermo Fisher Scientific, U.S.A). The following antibiotics: gentamicin (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), amikacin (30 µg), novobiocin (30 µg), ertapenem (10 µg), meropenem (10 µg), cefepime (30 µg), ofloxacin (5 µg), ciprofloxacin (5 µg) and sulphamethoxazole (25 µg), (Oxoid Ltd, Basingstoke, United Kingdom) were placed on the inoculated plates. The Muller – Hinton agar plates were allowed to stand for 5 – 10 mins for proper diffusion and incubated at 37 ^{o}C in an aerobic atmosphere for 24 h. The clear zones of inhibitions were recorded using meter rule [8].

Determination of Multiple Antibiotic Resistance Index (MARI)

Multiple antibiotic resistance index(MARI) was determined using the formula MAR=a/b, where **a** is the number of antibiotics to which test isolate displayed resistance and **b** is the total number of antibiotics to which the test organism has been evaluated for sensitivity [9.]

RESULTS

Prevalence of *Escherichia coli* and *Klebsiella pneumoniae* among Neonates in Nursery Unit Mile Four Hospital Abakaliki

A prevalence rate of 9 (11.5 %) and 5 (6.4 %) were showed for male stool samples for *E. coli*. *K. Pneumonia* respectivelyand for female stool samples a prevalence rate of 5 (6.4 %) for *E. coli* and 7 (9.0 %) for *K. Pneumonia*.

Blood samples 11 (9.0 %) for males, 8 (6.6 %) for females were the prevalence rate for *E. coli,* while 7 (5.7 %) and 9 (7.4 %) were the prevalence rate of *K. pneumoniae* for both male and females respectively (see table 1 below).

Table 1: Prevalence of *Escherichia coli* and *Klebsiella pneumoniae* among Neonates in Nursery Unit Mile Four Hospital Abakaliki

Gender (%)		***E. coli* (%)**	***K. pneumoniae* (%)**
Stool samples	Male 42 (53.8)	9 (11.5)	5 (6.4)
	Female 36 (46.2)	5 (6.4)	7 (9.0)
Total 78 (39)		**14 (18.0)**	**12 (15.4)**
Blood samples	Male 67 (55.0)	11 (9.0)	7 (5.7)
	Females 55 (45.1)	8 (6.6)	9 (7.4)
Total	**122 (61)**	**19 (15.6)**	**16 (13.1)**
Overall Total	**200 (100)**	**33 (16.5)**	**28 (14)**

Demographic Factor of Newborns in Nursery Unit Mile Four Hospital, Abakaliki

The age and gender distribution of *E. coli* and *K. pneumoniae* which was isolated from newborns in Nursery Unit Mile Four Hospital, Abakaliki (Table 2 below). In which among ages 0 – 10 days *K. pneumoniae* and *E. coli* recorded 62 (62 %) predominating ages 11 – 21 days 26 (26 %), while the least occurrence rate of *K. pneumoniae* and *E. coli* 12 (12 %) was seen among the ages 21 – 28 days. Gender distribution of *E. coli* and *K. pneumoniae* revealed high occurrence rate of 57 (57 %) in male patients over females that recorded 43 (43 %).

Table 2: Demographic Factor of newborns in Nursery Unit Mile Four Hospital, Abakaliki

Variables		**No. Sampled**	***E. coli* and *K. pneumoniae***		**Total Isolates(%)**
Age (by days)	0 – 10	87	47	30	87 (43.5)
	11 – 20	69	42	27	69 (34.5)
	21 – 28	44	26	18	44 (22)
Gender	Female	91			91 (45.5)
	Male	109			109 (54.5)
Total		**200**			**200 (100)**

Distribution of *Escherichia coli* and *Klebsiella pneumoniae* isolated from different stool swab and Blood samples from Nursery Unit Mile Four Hospital, Abakaliki.

Distribution of *E. coli* and *K. pneumoniae* in different stool and blood samples from newborns in Nursery unit Mile Four Hospital, Abakaliki is shown in table 3. *E. Coli* and *K. Pneumoniae*ac counted overall occurrence rate of 200 (100 %) comprising of high isolation rate of 14 (7 %) for *E. coli*and12 (6 %) for *K. pneumoniae* in stool samples, while 19 (9.5 %) for *E. coli* and 16 (8 %) were for blood samples (Table 3).

Table 3: Distribution of *E. coli* and *K. pneumoniae* isolated from different stool and blood samples from Nursery Unit Mile Four Hospital, Abakaliki.

Clinical Samples	**No. of Samples (%)**	**No. of *E. coli* (%)**	**No. of *K. pneumoniae* (%)**
Blood	122 (61)	19 (9.5)	16 (8)
Stool	78(39)	14 (7)	12 (6)
Total	**200 (100)**	**33 (16.5)**	**28 (14)**

Antibiotic Susceptibility Profile of *Klebsiella pneumoniae* isolates from stool and blood samples collected from Nursery Unit Mile Four Hospital, Abakaliki

In this table 4, it showed antibiotic susceptibility profile of *K. pneumoniae* isolated from stool and blood samples of patients in Nursery Unit Mile Four Hospital, Abakaliki. *K. pneumoniae* were more susceptible to Gentamicin 100 %, Ofloxacin 100 % and Ciprofloxacin 100 % but was resistant to Amikacin 100 %, Ertapenem 100 %, Ceftazidime 100 %, Sulphamethoxazole92.9 % and Novobiocin 85.7 %.

Table 4: Antibiotic Susceptibility Profile of *Klebsiella pneumoniae* isolates from stool and blood samples collected from Nursery Unit Mile Four Hospital, Abakaliki

Antibiotics (µg)	**Stool**		**Blood**	
	R (%)	**S (%)**	**R (%)**	**S (%)**
Amikacin (30)	12 (100)	0 (0.0)	16 (100)	0 (0.0)
Ceftazidime (30)	12 (100)	0 (0.0)	16 (100)	0 (0.0)
Cefepime (30)	12 (100)	0 (0.0)	16 (100)	0 (0.0)
Ceftriaxone (30)	12 (100)	0 (0.0)	16 (100)	0 (0.0)
Ciprofloxacin (5)	0 (0.0)	12 (100)	0 (0.0)	19 (100)
Ertapenem (10)	12 (100)	0 (0.0)	16 (100)	0 (0.0)
Gentamicin (30)	0 (0.0)	12 (100)	0 (0.0)	19 (100)
Meropenem (10)	12 (100)	0 (0.0)	16 (100)	0 (0.0)
Novobiocin (30)	8 (66.7)	4 (33.3)	16 (100)	0 (0.0)
Ofloxacin (5)	0 (0.0)	12 (100)	0 (0.0)	12 (100)
Sulphamethoxazole (25)	10 (83.3)	2 (16.7)	14 (87.5)	2 (12.5)

Keys: n – number of isolates: Stool (12), Blood (16); R – Resistance, S – Susceptible

Antibiotic Susceptibility Profile of *Escherichia coli* isolates from stool and blood samples collected from Nursery Unit Mile Four Hospital, Abakaliki

This table 5, displays the antibiotic susceptibility profile of *Escherichia coli*isolatedfrom stool swab and blood samples of patients in Nursery Unit Mile Four Hospital, Abakaliki. *E. coli* were more susceptible to Ofloxacin 100 %, Gentamicin 100 %, Ciprofloxacin % but resistant to Ceftriaxone 100 %, Amikacin 100 % and Meropenem 100 %.

Table 5: Antibiotic Susceptibility Profile of *Escherichia coli* isolates from stool and blood samples collected from Nursery Unit Mile Four Hospital, Abakaliki

Antibiotics (µg)	**Stool**		**Blood**	
	R (%)	**S (%)**	**R (%)**	**S (%)**
Amikacin (30)	14 (100)	0 (0.0)	19 (100)	0 (0.0)
Ceftazidime (30)	14 (100)	0 (0.0)	19 (100)	0 (0.0)
Cefepime (30)	14 (100)	0 (0.0)	19 (100)	0 (0.0)
Ceftriaxone (30)	14 (100)	0 (0.0)	19 (100)	0 (0.0)
Ciprofloxacin (5)	0 (0.0)	14 (100)	0 (0.0)	19 (100)
Ertapenem (10)	14 (100)	0 (0.0)	19 (100)	0 (0.0)
Gentamicin (30)	0 (0.0)	14 (100)	0 (0.0)	19 (100)
Meropenem (10)	14 (100)	0 (0.0)	19 (100)	0 (0.0)
Novobiocin (30)	14 (100)	0 (0.0)	19 (100)	0 (0.0)
Ofloxacin (5)	0 (0.0)	14 (100)	0 (0.0)	19 (100)
Sulphamethoxazole (25)	10 (71.4)	4 (28.6)	15 (78.9)	4 (21.0)

Keys: n – number of isolates: Stool (14), Blood (19)
R – Resistance, S - Susceptible

Multiple Antibiotic Resistance Index (MARI) of resistant *Escherichia coli* and *klebsiella pneumoniae* isolated from patients in Mile Four Hospital Abakaliki

The Mean Multiple Antibiotic Resistance Index of resistant *E. coli* and *Klebsiella pneumoniae* isolated from patients in Nursery Unit Mile Four Hospital, Abakaliki.

Table 6: Multiple Antibiotic Resistance Index (MARI) of resistant *Escherichia coli* and *Klebsiella pneumoniae* isolated from patients in Mile Four Hospital, Abakaliki

Isolates	**MARI**
Escherichia coli	0.7
Klebsiella pneumoniae	0.7
MEAN	**0.7**

DISCUSSION

This investigation found two types of bacteria, specifically *Escherichia coli* and *Klebsiella pneumoniae. E. coli and K. pneumoniae*, which are significant clinical pathogens, have been discovered to be linked to infections in neonates and various pathological conditions. Their presence in infections occurring in newborns confirms findings from previous investigations that have been documented [10]. Additional investigation of these bacteria may also reveal more genera linked to illnesses in babies. However, the percentage rate of *E. coli* in both blood (9.5%) and stool (7%) was higher than the previously reported data from neonate samples in Nigeria. The previous data showed a proportion rate of 7.7% for *E. coli* in blood samples and 8% for *K. pneumoniae,* and 6% for E. coli in stool samples. A very high prevalence of *E. coli* and *K. pneumoniae,* accounting for 43.5% of cases, was

observed in the age group of 0-10 days. Conversely, the lowest occurrence rate of both *E. coli* and *K. pneumoniae,* representing 22% of cases, was reported among individuals aged 21-28 days. Furthermore, the levels of *E. coli* and *K. pneumoniae* were found to be greater than those reported in a prior study conducted by Ojukwu *et al*[11]. The observed variability in the frequency of *E. coli* and *K. pneumoniae* among different age groups may be attributed to factors such as the study's design (including the age range, characteristics of the population investigated, severity of infection, and sample size) that were takeninto account throughout the recruitment process. It is important to acknowledge that the research being referred to utilised varying age categories, so the comparisons are made at a broader or more general level.

The gender of the patient was found to be correlated with the presence of increased colonization of *E. coli* and *K. pneumoniae* in males (109, 54.5%) compared to females (91, 45.5%). However, other researchers have also observed a similar pattern. For instance,[12] reported a frequency of isolation in males (120, 45.5%) and females (49, 34.5%). From a scientific perspective, the higher colonisation of *K. pneumoniae* and *E. coli* in males can be attributed to the significant number of male patients who were hospitalised in the study area at the time of this research.

Out of the clinical samples, *E. coli* exhibited a prevalence of 33 cases (16.5%), whereas *K. pneumonia* had a prevalence of 28 cases (14%). This observation implies that the choice of antibiotic used during therapy can decrease the likelihood of colonisation by *E. coli* and *K. pneumoniae*. The rise in the occurrence of *E. coli* and *K. pneumoniae* can be attributed to the lack of proper hygiene and inadequate treatment within the population being investigated, which has resulted in the rapid growth of these bacteria.

Escherichia coli 33 has the highest incidence rate (16.5%) among the samples in this investigation. *Escherichia coli,* a Gram-negative bacterium that is capable of movement, is commonly present in the intestinal system and has been linked to the development of diarrheal illnesses. The organism is highly pathogenic and commonly found in the urinary system of humans. It is known to cause infections in wounds, meningitis, and bacteremia in neonates [13].

Klebsiella pneumoniae was the least prevalent bacteria, with a recorded isolation rate of 28 (14%). *Klebsiella pneumoniae* is commonly present as part of the natural microbial community in the mouth, skin, intestines, and as saprophytes in soil and water. Nosocomial infections have increasingly become a significant cause, as highlighted by Driscoll *et al*[14.]

The isolates obtained from the samples collected in this investigation confirm the role that these organisms play in the transfer of illnesses from mother to child. The study found that both E. coli and K. pneumoniae isolates were completely susceptible to Gentamicin (100%), Ofloxacin (100%), and Ciprofloxacin (100%). These results align with the findings of Driscoll [14,] who reported a high susceptibility of E. coli to Ciprofloxacin (85%) and Gentamicin (90%).

E. coli and *K. pneumoniae* exhibited complete resistance to cephalosporins, with a resistance rate of 100%. These findings are consistent with a previous study conducted in Abakaliki, where *E. coli* showed resistance to ceftazidime (89.7%) and ceftriaxone (58.6%), as reported by Ejikeugwu and Amaechi [16]. Similarly, Yardav and Parkash [17] observed the highest resistance to ceftriaxone (67.47%) and ceftazidime (63.41%) in the Southern Terai region of Nepal. In a study conducted by Fernando *et al*[18]in Sri Lanka, the highest levels of resistance were seen with ceftriaxone (100%) and ceftazidime (100%). In previous research, *K. pneumoniae* isolates demonstrated high levels of resistance to ceftriaxone (100%), ceftazidime (95.8%), and cefepime (83.3%) [19]

A previous investigation conducted in Saudi Arabia found that four isolates of ESBL *K. pneumoniae* exhibited complete resistance (100%) to cefepime, ceftriaxone, and ceftazidime

[20]. The high rate of resistance observed in this study can be attributed to the indiscriminate utilisation of cephalosporins and Beta-lactam antibiotics by individuals. This has posed a problem in treating microbial infections and diseases caused by these antibiotic-resistant organisms due to the production of ESBL. AmpC β-lactamases are enzymes found in organisms that do not have highly active chromosomal AmpC genes, such as *K. pneumoniae and E. coli*. The resistance pattern identified in this study, where third generation cephalosporin is resisted by 50-100%, may be caused by the overexpression of AmpC β-lactamases encoded on the chromosomes [21].

Previous research has documented the existence of plasmid mediated quinolone resistant (PMQR) genes that are transported by the ESBL-producing plasmid. This plasmid enables the development of greater resistance to quinolone medications. These findings were reported by Onyenwe et al [22] and Riyaaz *et al*[23].

The strain exhibited multidrug resistance, as evidenced by a MARI value of 0.7, in both *K. pneumoniae* and *E. coli* from several samples. The study by Thenmozhi*et al*[24] found that MARI index values more than 0.2 suggest a high probability of contamination from sources where antibiotics are often utilised. The Neopane*et al*[25] study has revealed the clinical efficacy of these medicines in the treatment of infections caused by multidrug-resistant bacteria.

CONCLUSION

This study demonstrates that the prevalence of *E. coli* in neonate infections is higher, accounting for 33 cases (16.5%), compared to *K. pneumoniae*, which accounted for 28 cases (14%). This situation presents possible public health implications for healthcare professionals, patients, and individuals, as *E. coli and K. pneumoniae* are formidable pathogens capable of causing infections, particularly in children, especially among neonatal patients. It is crucial that these pathogens are addressed seriously and treated appropriately. *Klebsiella pneumoniae* and *Escherichia coli* exhibited greater susceptibility to fluoroquinolones, suggesting that these antibiotics can be regarded as the preferred treatment option. Pathogens can be resistant to infections in infants and may lead to treatment ineffectiveness. Therefore, it is necessary to implement an antimicrobial stewardship programme and conduct regular testing of infants and pregnant women in order to promptly intervene. This study urges healthcare professionals, particularly those in newborn care units, to raise awareness among expecting mothers about the possible risks of infections linked with contact to these microorganisms.

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