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Antibiogram of Bacterial isolates obtained from Infants and Children at the University of Calabar Teaching Hospital (UCTH).

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Abstract

The gradual increase in antimicrobial resistance in children is a major challenge in the field of hospital epidemiology and medical microbiology. This study was conducted to investigate the susceptibility pattern of commonly used antimicrobial agents against bacterial isolates in children attending UCTH. Fifty clinical specimens; blood, urine, eye, ear and wound swap were collected from infants and children 1-day old to 9 years. About 2mL of the blood was dispensed into Brain Heart infusion broth (10ml) contained in a Bijou bottle and incubated for 2 days at 37^{0}C. Midstream urine samples were collected into sterile universal containers and cultured on blood and CLED agar using the Leigh and Williams paper strip method, while sterile swab-stick was used to collect ear and eye specimen (discharge). The ear specimens were cultured on CLED and blood agar while the eye swap on CLED and chocolate agar. Isolates susceptibility was tested on gentamicin, erythromycin, Zithromax, ampicillin, amoxicillin and penicillin using disc diffusion method by Kirby-Bauer. Of the 50 subjects examined, a total of 39 bacterial isolates were obtained from the 36(72%) culture positive samples. *S. aureus* was the most isolated 22(56.4%), followed by *P. aeruginosa 7*(17.9%), *Streptococci, Coliform*, and *Proteus* were 3(7.7%), 6(15.4%) and 1(2.6%) respectively. *F*emale children had more bacterial isolates 19(73.1%) compared to male 17(70.1%). The isolates were more sensitive to gentamicin 25(64%), followed by zithromax 23(59%), erythromycin 16(41%) and ampicillin 12(31%). The isolates were less sensitive to Penicillin 2(5) and Amoxicillin 10(26), multi resistance was seen in 14(35.9%) of the isolates. Resistance to antibiotics is on the rise and babies are particularly at increased risk because of lower immunity. The study recommends the need for the development of alternative therapy.

Keywords: Infection, Bacterial isolates, Neonates, Antibacterial, Resistance, Sensitive

Introduction

Over the last decade, programs targeting specific childhood illness such as diarrhea and acute respiratory tract infections along with higher immunization coverage and better management of illness in children have contributed to significant decline in infant mortality rates in many developing countries. However, one of the major challenges in the field of hospital epidemiology is the continuous increase in antimicrobial resistance (Lagunju *et al*., 2008; Akingbad *et al*., 2013).

Therapy of infectious diseases caused by bacteria resistance to multiple antimicrobial agents had emerged as one of the greatest challenges facing clinicians. Problems created by antibiotic resistance have profound effects upon the practice of Paediatrics resulting in a continuing need for revision of recommended therapies of common infections. Every year, an estimated 30 million newborns acquire a neonatal infection and 1-2 million neonates die from infection. These infections include tetanus, sepsis and acute respiratory tract infections.

The umbilical cord is a major route of infection in the early neonatal period accounting for the majority of neonatal infection and many cases of neonatal sepsis. Children can also acquire

infection accidentally during a stay in the hospital (Nosocomia infections) (WHO, 1991; Luke *et al*., 2003; Amare, 2014, Coffey & Brown 2017). By this, it is possible for a child to acquire more infection other than that in which the child was admitted in hospital for. These infections posed a significant threat to the hospitalized child and will continue to be a cause of significant morbidity and mortality. Some bacteria commonly isolated include; *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis* etc. Infection can as well be acquired outside the hospital environment, and this is known as community acquired infection. These infections are caused by organisms that are highly prevalent in the community e.g. *Vibrio cholera, Salmonella typhii, Streptococcus pneumonia, Staphylococcus aureus* etc. (Richard, 1987; Martin *et al*., 2005; Anthony *et al*., 2009).

Resistance to most first-line antibiotics has increased over the past decades, this poses a challenge to the health system. Several factors have contributed to increase in antimicrobial resistance, notably are, antibiotics abuse, fake (unlicensed drug producers), the use of antibiotics in poultry and livestock feed in a minor concentration in order to promote growth, international travel and increased importation of food. These factors are responsible for the spread of antibiotic resistant bacteria between developed and developing countries (Pediatric and Child Health, 2003).

Resistance to antimicrobial agents can be acquired through any of the following three mechanisms: bacteria natural resistance, genetic mutation, or by transfer of genetic material from one organism to another even among those of different species (Willey *et al.*, 2008).

Aim and Objectives

The aim of this study is to investigate the susceptibility pattern of commonly used antimicrobial agents to nosocomia and community isolates in children at the UCTH. The objectives of the study include:

i. To determine the prevalence of bacterial infection in children and infants receiving treatment at the UCTH
ii. Isolate and identify common bacterial pathogens associated with neonatal infection.
iii. To test the susceptibility pattern of the isolates to commonly used antimicrobial agents.

Materials and method

The study subjects

The study subjects included children attending University of Calabar Teaching Hospital and was conducted in the special care baby Unit (SCBU), sick baby unit (SBU), children emergency unit (CHER) and children out-patient unit (CHOPD). SCBU admits babies referred from outside hospital born within 48 hours. CHOPD admits older children. Subjects included in this study were aged 1-day old to 9 years. Samples were also collected from children who were on admission (in-patients).

Study population/sample size

A total of 50 subjects were sampled from full-term babies, infants and children admitted into SBU, SCBU, CHOPD and CHER.

Sample collection and Processing

Materials used for collection and isolation of various bacterial isolates include;

- Two milliliters of venous blood were collected and dispensed into Brain Heart infusion broth (10ml) contained in a bijou bottle, incubated for 2 days at 37^0C.
- Midstream urine samples were collected into sterile universal containers and cultured on blood agar (BA), and Cysteine Lactose Electrolyte Deficient (CLED) agar using the Leigh and Williams paper strip method.
- Sterile swab-stick was used to collect ear and eye specimen (discharge); ear specimens were cultured on CLED and blood agar; eye swap on CLED and chocolate agar. Nutrient agar was used for disc diffusion sensitivity testing.
- Microscopic examination; wet preparation was done using the centrifuged deposited of the urine.

Isolation and identification of organism

Isolates were identified on the basis of their cultural characteristics and Gram staining reaction. They were also subjected to biochemical test such as oxidase, catalase and coagulase test. Commercially prepared antibiotics were used, which include Zithromax (Z), Erythromycin (E), Ampiclox (AMP), Amoxicillin (AM), Gentamicin (CN) and Penicillin (P).

Results

A total of 39 bacterial isolates were obtained from the 36 culture positive samples, of this, *S. aureus* was the most isolated 22(56.4%), followed by *P. aeruginosa* 7(17.9%), Streptococci, Coliform, and Proteus were 3(7.7%), 6(15.4%) and 1(2.6%) respectively.

From the 50 subjects sampled, 36(72%) showed significant bacterial growth with a total of 39 bacterial isolates, while 14(28%) showed no bacterial growth. Of the total 39 bacterial isolates obtained from the 36 culture positive samples, *S. aureus* was the most isolated 22(56.4%), followed by *P. aeruginosa* 7(17.9%), *Streptococci*, *Coliform*, and *Proteus* made up of 3(7.7%), 6(15.4%) and 1(2.6%) respectively (Figure 1).

With the exception of wound swab, *S. aureus* was isolated from all other four clinical samples; *P. aeruginosa* was not isolated in wound and blood samples, but present in all other specimens. Other Coliforms were recovered from blood and urine specimen only, while *Streptococcus species* were isolated from blood and wound swap (Table 1).

Table 2 shows age and gender distribution of the study subjects. By gender, female children had higher prevalence of bacterial isolates 19(73.1%) compared to male 17(70.1%).

From the total 10 children; 4 males and 6 female aged 2-5 years examined, 3(75%) and 5(83%) were culture positive respectively. Of the eleven children (8 males and 3 female) studied, 5(62.5%) of the male, and 2(66.7%) of the female showed positive culture.

The isolates were more sensitive to gentamicin 25(64%), followed by zithromax 23(59%), erythromycin 16(41%) and ampicillin 12(31%). The isolates were less sensitive to Penicillin 2(5) and Amoxicillin 10(26). Sensitivity profile of the isolates showed that *Streptococcus species* were more sensitive to the antibiotics used (55.7%), followed by *Staphylococcus aureus* (48.4%). Sensitivity profile of *Proteus, Coliforms* and *P. aeruginosa* was 16.7% each respectively (Table 3).

Staphylococcus aureus was 16(72.3%) sensitive to Zithromax, 12(54.5%) sensitive to erythromycin, 10(45.5%) sensitive to ampicillin, 8(36.4%) to amoxicillin, 16(72.2%) to gentamicin and 2(9.1%) to penicillin. *Pseudomonas aeruginosa* was 1(100%) sensitive to gentamicin, but showed 0% sensitive to zithromax, erythromycin, ampicillin, amoxicillin and penicillin. Coliforms were 4(36.4%) sensitive to zithromax, 6(54.5%) to gentamicin, 0(0%) to erythromycin, ampicillin, amoxicillin and penicillin. *Streptococcus specie* showed 3(100%) sensitivity to Zithromax and erythromycin, 2(66.7%) to ampicillin and amoxicillin, 0% to gentamicin and penicillin respectively. *Proteus* showed 2(100%) sensitivity to gentamicin but was 0(0%) resistant to zithromax, erythromycin, ampicillin, amoxicillin and penicillin respectively. Antibiotics susceptibility pattern of nosocomial and community isolates is shown on table 3.

Table 1: Distribution of isolates by specimen type

Specimens type	Number of specimens	Bacteria isolated
Blood	30	*Streptococcus species* *Other Coliforms* *Staphylococcus aureus* *Coagulase negative staphylococcus*
Eye swabs	6	*Pseudomonas aeruginosa* *Staphylococcus aureus*
Ear swabs	5	*Proteus species* *Pseudomonas aeruginosa* *Staphylococcus aureus*
Wound swab	1	*Streptococcus species*
Urine	4	*Other Coliforms* *Pseudomonas species* *Staphylococcus aureus*

Table 2: Age and gender distribution of children with positive cultures

	Male		Female	
Age	**Total number**	**Positive number**	**Total number**	**Positive number**
< 1 year	12	9(75%)	17	12(70.6)
2-5 year	4	3 (75%)	6	5(83.3)
6-9 year	8	5 (62.5%)	3	2(66.7)
Total	**24**	**17(70.1%)**	**26**	**19(73.1%)**

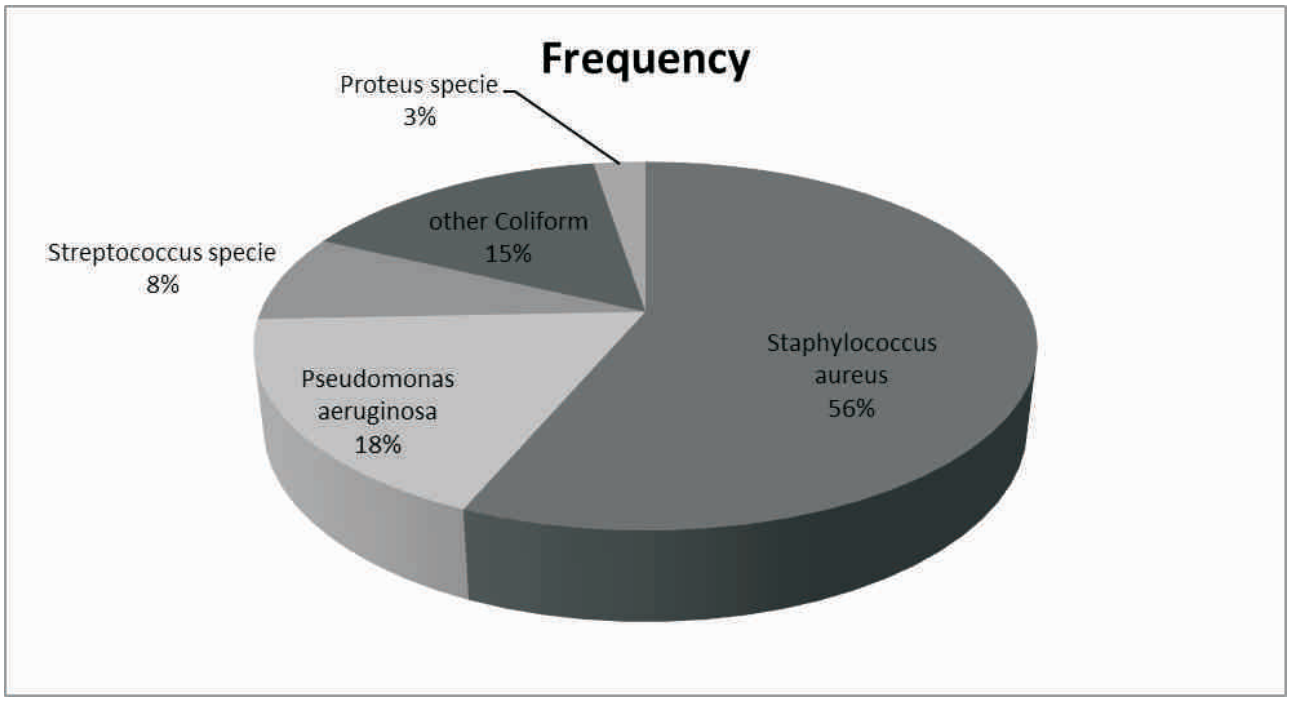

Figure 1: Frequency of occurrence of bacterial isolates

Table 3: Antibiotic sensitivity pattern of community isolates

Organisms	**No of isolates**	**Antibiotic sensitivity (%)**						
		Z	**E**	**CN**	**AM**	**AMP**	**P**	**Average (%)**
S. aureus	22	16(72.3)	12(54.5)	16(72.3)	8(36.4)	10(45.5)	2(9.1)	48.4
Streptococcus species	3	3(100)	3(100)	0(0)	2(66.7)	2(66.7)	0(0)	55.7
Other Coliforms	11	4(36.4)	1(9)	6(54.5)	0(0)	0(0)	0(0)	16.7
Proteus	2	0(0)	0(0)	2(100)	0(0)	0(0)	0(0)	16.7
P. aeruginosa	1	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	16.7
Total Isolates	39	23(59)	16(41)	25(64)	10(26)	12(31)	2(5)	

Key: Z=Zithromax, CN=Gentamicin, AMP=Ampicillin, E=Erythromycin, AM= Amoxicillin, P=Penicillin

Discussion

Infants aged 0-1 year, had more cases of bacterial infection compared to older children. Though the reason for this is unknown; it may probably be due to stronger immunity of the older children. At birth to early months of birth, the baby's immunity mostly dependent on natural passively acquired immunity from the mother (IgG that crosses the placenta and IgA secreted in breast milk). As the child grows, it begins developing its immunity and becomes stronger. (Prescott *et al*., 2008). Infections in these infants must have been acquired either through the hospital staffs who attend to the babies, from the mother or the environment. Van den Bruel *et al*. (2006) observed that the prevalence of neonatal sepsis is mostly in the first month of life, and the prevalence rate of bacterial infection diminishes with increase in age, because older children have a better developed immune system (American Academy of Family Physicians, 2021; *Geoffrey, 2021*). Higher prevalence of bacterial infection in younger babies have been well reported (Sherman *et al*., 2011; Chipwaza *et al*., 2015; Brett *et al*., 2021).

Female children had higher cases of bacterial isolates than male 73.1% vs 70.1%. This corroborates the findings of Pamela (1971) and Zenoh *et al*. (2018) where he reported more cases in adult female than male. Others on the other hand, reported higher prevalence in male than female (Chipwaza *et al*., 2015; Aghai *et al*., 2020; Pathak, 2020; Brett *et al*., 2021).

Staphylococcus aureus was the most isolated bacterial pathogen, followed by Pseudomonas, coliforms *Streptococcus specie and proteus* had the least occurrence. Since neonates acquired infection either from the medical staffs, or from the baby's parent, it is ideal for *S. aureus* (normal flora of the skin) to be the most isolated pathogen. Pathak (2020) in his study of the incidence, clinical profile, and risk factors for serious bacterial infections in children hospitalized with fever in Ujjain India, isolated *Staphylococcus aureus* as the most common gram-positive bacteria, and *Escherichia coli* as the most isolated Gram-negative organism. Alison *et al*. (2010) in contrast, reported Group B *Streptococcus as the most isolated organism, followed by Acinetobacter spp*., and *Escherichia coli.* Karin (1999) also recovered group B *Streptococcus* 14(11.6%) as the most isolated, followed by *Enterococcus* 7(5.8%), *S. pneumoniae* 3(2.5%), and *N. meningitidis* 1(0.8%). Brenda (2020), recovered group B *Streptococci*, *Escherichia coli*, *Listeria monocytogenes*, gonococci, and *Chlamydia*. Itzhak (2003), also isolated group B Streptococcus, Escherichia coli, Listeria monocytogenes H. influenzae, S. aureus, N. meningitidis, and Salmonella spp.

Gentamicin was the most effective antibiotic with activities in over 25(64%) of the 39 bacterial isolates, this is followed by zithromax and erythromycin exhibiting activities in 23(59%) and 16(41%) of the isolates respectively. However, the isolates were less sensitive to the beta lactam ring antibiotics with over 37(95%) resistant to penicillin.

Alison *et al. (*2010) in contrast reported that the beta lactam ring antibiotics shows greater activities with over 81% of the bacterial isolates susceptible to penicillin and/or gentamicin and 84% to ampicillin and/or gentamicin. Adejumoke *et al*. (2021) studied the management of possible serious bacterial infection in young infants where referral is not possible in the context of existing health system structure in Ibadan, South-West Nigeria, and oral amoxicillin shows sensitivity in 312(80%) of 390 bacterial isolates.

Pokhrel, *et al*. (2018) isolates showed high resistance to commonly used antibiotics such as; Cefotaxime (90.5%), Gentamicin (75%), Ciprofloxacin (76.2%), Ofloxacin (72.2%) and Chloramphenicol (65%), with 73.9% multidrug-resistant cases.

Byungwoo, *et al*. (2019) observed that antimicrobial susceptibility to ampicillin was steadily low and to amikacin was consistently high for both periods. Dustin *et al*. (2017) observed among their cohort that isolates were resistant to ampicillin with susceptibility rate of 83.2% to aminoglycosides. Like this finding, Li *et al*. (2019) also recorded high cases of resistance to the beta lactam ring antibiotics particularly penicillin and ampicillin though

sensitive to amikacin and imipenem. Most isolates of Gram-positive bacteria were sensitive to vancomycin, linezolid, minocycline and tigecycline. *Streptococcus* and *S. aureus* were the most susceptible isolates with average susceptibility of 55.7% and 48.4% respectively. Eighty-four percent each of o*ther Coliforms, Proteus and P. aeruginosa were resistant to the isolates used.*

Conclusion

Staphylococcus aureus is the major bacterial pathogen isolated from babies and children in UCTH *in this age group, followed by the Coliform.* Gentamicin and zithromax were the most effective drug against neonatal isolates. Isolates shows great resistance to the beta lactam ring antibiotics such as amoxicillin, ampicillin and penicillin.

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