CLINICAL PROFILE, COMMON BACTERIAL ISOLATES AND ANTIMICROBIAL SUSCEPTIBILITY TEST AMONG NEONATES WITH SPINA BIFIDA ADMITTED AT MUHIMBILI NATIONAL HOSPITAL.

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ABSTRACT

Background

Open spina bifida is associated with bacterial infection including: meningitis, and severe sepsis. Despite empirical initiation of first or second line antibiotics to most neonates on admission sepsis is still a major problem.

Objective

To describe the clinical profile, common bacterial isolates and antimicrobial susceptibility patterns among neonates with infected spina bifida lesions admitted at Muhimbili National Hospital.

Methodology

A hospital based descriptive cross sectional study was conducted among 52 consecutively recruited neonates with spina bifida admitted at neonatal unit from November 2019 to April 2020. A structured questionnaire was used to collect information on demographic characteristics. Physical examination was done to describe the clinical profile of the spinal bifida and swabs were collected for bacterial culture and antibiotic susceptibility testing from all enrolled neonates with open lesions.

Results

Majority of the participants (90.4%) had myelomeningocele and most defects (42.3%) were located on the lumbar spine, (57.7%) consisting of intact cystic lesions, with an average diameter of 3-5 cm amongst 63.5% of the participants; and 31(59.6%) of the lesions had necrotic tissues. Majority of the neonates 44(84.6%) were started on empirical antibiotics on admission, 80.8% being either ampicillin-cloxacillin/gentamycin or ceftriaxone. Most of the swabs 40(85.1%), cultured had bacteria growth, about two thirds of the bacteria isolated 23(70%) were gram negative

with a predominance of *Klebsiella spp* 10 (25%) which showed good sensitivity of ≥80% to amikacin and meropenem but had high resistance to ceftriaxone (90%), amoxclavulinic acid (80%), tazocin (70%), and ciprofloxacin (60%); common gram positive bacteria isolated was *Staphylococcus aureus* 6 (15%) with high sensitivity to meropenem (83%) and high level of resistance other antibiotic tested including vancomycin (50%). The likelihood of isolating bacteria from spina bifida lesions was significantly higher in neonates who had associated bladder-bowel incontinence (p=0.025).

Conclusion

Majority of the participants had open, medium to large lesions located in the lumbar area, 40% of which had ruptured resulting in a very high bacterial colonisation rate of >85%. There was very low threshold (9.1%) for prenatal diagnosis of spina bifida by obstetric ultrasound. Most spina bifida defects were colonised by gram negative bacteria commonly *Klebsiella spp* sensitive to meropenem and amikacin, but resistant to the first and second-line antibiotics recommended in our national guidelines. Neonates who had coexisting bladder-bowel incontinence were more likely to have the lesions colonised by bacteria.

Introduction

Spina bifida constitutes of more than 76% of all NTD admissions at Muhimbili National Hospital (MNH) (1) and is associated with infection including: meningitis, and severe sepsis, leading to further neurological damage, and prolonged hospital stay. All neonates with myelomeningocele would benefit from sterile management and closure of the defects within 48 hours of birth (2) which remains a significant challenge in our settings. More than half of perinatal deaths encountered among the neonates with external structural birth defects at Muhimbili Hospital are associated with neural tube defects including spina bifida (3), a birth defect with well-established evidence based preventive interventions (4). Prenatal diagnosis of spina bifida is now possible using obstetric ultrasound which is cheaper, non-invasive and widely accessible in most part of the world. But in developing countries there is very low prenatal diagnosis that could not only be useful to provide better counseling to the expectant mothers but also facilitate early transfer to centres with neurosurgical services for early surgical closure of the defects (5, 6). Whilst it is standard practice to administer prophylactic antibiotics, there is little evidence to support this

practice in ideal settings where surgical closure of the defect is likely to occur within 48 hours (7). However, in settings like ours where there is late presentation and minimal resources to ensure complete sterile management, there is still a role for prophylactic antibiotics. High levels of resistance to first and second line antibiotics used in our setting has been reported in the Lancet (2013) (8), which poses the need to do culture and AST to rationalize the use of antibiotics. This study aimed to inform clinicians on clinical presentations, common local bacteria isolates from open spina defects and recommended treatment options based on antimicrobial susceptibility test in our setting. Clinicians will therefore provide evidence-based care, improve the quality of life of the affected neonates and reduce the neonatal morbidity and mortality contributed by sepsis at Muhimbili Hospital.

Method

Study design and participants

This was a hospital based descriptive cross sectional study, conducted over a period of six months from November 2019 to April 2020. All neonates with spina bifida admitted at MNH neonatal unit during the study period were included except those whose mother(s) did not give consent to participate in the study. Sample size of this study was estimated at 52 by using an adjusted formula for a finite population size of 54 neonates with spina bifida in the neonatal unit for a period of six month and the proportion obtained from Brazilian study of 28.4% for infected spina bifida by pathogenic bacteria with a margin of error of 3% at the 95% confidence level.

Study setting

This study was conducted in the neonatal unit at Muhimbili National Hospital in Dar es Salaam Tanzania. The neonatal unit receives neonates delivered at MNH and those referred from the regional referral hospitals in Dar es Salaam region and those referred from other 31 regions of the country, including neonates with spina bifida. The unity has a bed capacity of 129 beds, with a minimum and maximum bed state of 100 to 180 respectively. The average number of admissions is approximately 15-25 neonates per day, with the total annual admissions estimated at 7500 neonates including at least 100 neonates with spina bifida.

Data collection

A standardized structured Swahili questionnaire was used as a data collection tool. Socio-demographic data and other relevant information were obtained through interviewing the mother and a thorough general and neurological assessment was done on the neonates to describe the state of the lesions either ruptured or intact and any ruptured lesion with cloudy drainage or intact lesion with necrotic part was considered infected. A swab was collected from the lesion of all enrolled neonates with open spina bifida 48 hours from birth for inborn and within 24 hours of admission from outborn neonates and samples were taken for bacterial culture and sensitivity test at the Central Pathology Laboratory at MNH within one hour.

Antimicrobial susceptibility test

This was done for antibiotics used in treatment of sepsis in our setting as dictated by the Tanzanian national treatment guidelines. Ampicillin and gentamycin as first line whilst the second line tested were ceftriaxone, vancomycin and amoxiclavulinic acid. The third line antimicrobials tested was meropenem. Drug concentrations that were used included; Ampicillin 10µg, gentamicin 10µgceftriaxone 30µg, vancomycin 30µg amoxiclavulinic acid 30µgm and meropenem 10µgm respectively. In addition, for cultures which had isolated gram negative bacteria such as *Klebsiella spp* and *Pseudomonas spp* the following antibiotics were added: amikacin 30µgm, ciprofloxacin 5µgm and piperacillin tazobactam100µgm/10µgm whilst for *Staphylococcal spp* addition of erythromycin 15µgm and clindamycin 2µgm was done to meet the Clinical Laboratory Standard Institute (CLSI) guideline. Drug concentrations that was used included; Ampicillin 10µg, gentamicin 10µg ceftriaxone 30µg, vancomycin 30µg amoxiclavulinic acid 30µgm and meropenem 10µgm. Sensitivity was tested using disc diffusion method, (Kirby- bauer) and sensitivity was based on the clinical and laboratory standard institute system (CLSI). The results were recorded as sensitive or resistant, if the zone of growth inhibition was less than what was expected from the disc diffusion for the particular tested antimicrobial.

Data analysis

Data were entered into statistical package for social science (SPSS) version 23. Categorical variables were analyzed using frequencies and proportions. Measure of association between the clinical profile and bacterial colonisation was done using Chi square or Fisher's exact test for categorical variable. A P-value of 0.05 or less was considered to be statistically significant.

Result

Demographic and clinical attributes of the participants

A total of 52 neonates were enrolled, majority were delivered by SVD 41(78.8%), at term 48(92.3%), with a normal birth weight 41 (78.8%) and there was male predominance 34 (65.4%).

Less than half of the neonates (44.2%) were admitted at MNH within 48 hours post-delivery because most of the participants (53.8%) were born outside of Dar es Salaam region. Although almost two thirds 33(63.5%) of the mothers had an antenatal screening ultrasound, only 3 (9.1%) of the spina bifida cases were diagnosed prenatally.

At enrollment only 15 (34.6%) of the neonates had fever, majority had no prior history of convulsion 94.2%, but nearly half 24(46.2%) of the neonates had a high level initial CRP of >10mg/L indicating possible bacterial infection. On admission majority of the neonates 44(84.6%) were started on empirical antibiotics, which consisted of either ampicillin-cloxacillin/gentamycin or ceftriaxone in up to 80.8% of the participants, as shown in Table 1.

Table 1: Demographic and clinical attributes of neonates with spina bifida at MNH (N=52)

Variable	Category	N (%)
Gestational Age	Preterm	4(7.7)
	Term	48(92.3)
Sex	Male	34(65.4)
	Female	18(34.6)
Birth weight	<2.5kg	5(9.6)
	2.5 -3.5kg	41(78.8)
	>3.5kg	6(11.5)
Place of birth	Within Dar Es Salaam	24(46.2)
	Outside Dar Es Salaam	28(53.8)
Prenatal USS	Yes	33(63.5)
	No	19(36.5)
Time of diagnosis	Prenatal (USS)	3(5.8)
	Postnatal (clinically)	49(94.2)

Mode of delivery	SVD	41(78.8)
	Cesarean section	11(21.2)
Duration since birth to admission	<2 days	23(44.2)
	2-7 days	18(34.6)
	>7 days	11(21.2)
Fever	Yes	18(34.6)
	No	34(65.4)
Convulsion	Yes	3(5.8)
	No	49(94.2)
Initial C-Reactive protein level	Normal (0-10mg/l)	18(34.6)
	High (>10mg/l)	24(46.2)
	Not done	10(19.2)
Empirical antibiotics initiated	Yes	44(84.6)
	No	8(15.4)
Choice of empirical antibiotic used	Ampiclox/Gentamycin	16(30.8)
	Ceftriaxone	26(50.0)
	Others*****	2(3.8)
	Not on antibiotics	8(15.4)

*****_One was on ciprofloxacin and the other one on meropenem._

Clinical profile of the neonates with spina bifida at MNH.

Majority of the participants (90.4%) had myelomeningocele. Most defects (42.3%) were located on the lumbar spine, (57.7%) consisting of intact cystic lesions, with an average diameter of 3-5 cm amongst 63.5% of the participants; and (59.6%) had necrotic tissues. The most common associated complications included: hydrocephalus (86.5%), bladder-bowel incontinence (86.5%), paraplegia (76.9%), and talipes equinovarus (17.3%). By the end of the six month study period, only 42.9% of those requiring surgery had surgical closure, as shown in Table 2.

Table 2: Clinical profile of neonates with spina bifida at MNH (N=52)

Variables	Category	N (%)
Type of Spina bifida	Myelomeningocele	47(90.4)
	Meningocele	5(9.6)
Site of the defect (SB)	Thoracolumbar	9(17.3)
	Lumbar	22(42.3)
	Lumbosacral	16(30.8)
	Sacral	5(9.6)
Appearance of the SB	Cystic ruptured	21(40.4)
	Cystic intact	30(57.7)
	Ulcer	1(1.9)
Size of the SB	1-3cm	1(1.9)
	>3-5cm	33(63.5)
	>5cm	18(34.6)
Condition of the SB	Clear CSF leakage	4(7.7)
	Discharging pus	6(11.5)
	Necrotic tissues	31(59.6)
	Clean	11(21.2)
TEV	Yes	9(17.3)
	No	43(82.7)
Fecal/urine incontinence	Yes	45(86.5)
	No	7(13.5)
Lower limbs movement	Normal	12(23.1)
	Weak	17(32.7)
	Absent	23(44.2)
Presence of hydrocephalus	Yes	45(86.5)
	No	7(13.5)
Surgical closure done	Yes	21(42.9)
	No	28(57.1)*****

*******Three of them did not require surgical closure.*

Common bacterial isolates among participants with open spina bifida.

Majority of the swabs 40(85.1%), cultured from the spina bifida defects had bacteria growth, whereby 35(87.5%) had one bacteria isolated and 5(12.5%) had two different bacteria isolated; which signifies a low level of contamination. Most of the bacteria isolated were gram negative 23(70%) with a predominance of *Klebsiella spp* 10 (25%); whilst the most common gram positive bacteria was *Staphylococcus aureus* 6 (15%) as shown in Table 3.

Table 3: Common bacterial isolates among neonate with spina bifida at MNH (N-52)

Variables	Categories	N (%)
Swab culture	Bacteria isolated	40(76.9)
	No Bacteria growth	7(13.5)
	Swab not done*****	5(9.6)
Number of bacteria isolated per swab culture	One bacteria	35(87.5)
	Two bacteria	5(12.5)
Common bacteria found		
Gram negative	Klebsiela species	10(25.0)
	Acinetobacter species	7(17.5)
	Escherichia coli	6(15.0)
	Pseudomona auriginosa	3(7.5)
	Morganella morganii	1(2.5)
	Proteus vulgaris	1(2.5)
Gram positive	Staphylococcus aureus	6(15.0)
	Staphylococcus albus	3(7.5)
	Bacillus species	3(7.5)

***Swab was not taken as the lesions had intact skin with no necrotic tissue.*

Antibiotic susceptibility patterns among bacteria isolated from neonates with spina bifida at MNH

Antibiotic resistance was variable across the bacterial isolates ranging from 0% to 100%. There was high level of resistance to commonly used first-line antibiotics such as gentamycin (29-100%) and second line antibiotics such as ceftriaxone (67-100%). The *Klebsiella spp* isolated in this study showed good sensitivity of ≥80% to amikacin and meropenem but had high resistance to ceftriaxone (90%), amoxclavulinic acid (80%), tazocin (70%), and ciprofloxacin (60%). This pattern was consistent amongst most gram negative bacteria isolated except that *Pseudomonas spp*, *Acinetobacter spp* and *Morganella spp* showed moderate susceptibility to ciprofloxacin.

On the other hand, the gram positive isolates were mainly *S. aureus* which showed a high sensitivity to meropenem (83%) but had a high level of resistance to all other antimicrobials including vancomycin (50%) as shown in Table 4**.**

Table 4. Antibiotics susceptibility test on isolated bacteria among neonates with SB at MNH (N=37)

BACTERIA	AMK S (%)	AMOC S (%)	CEF S (%)	CIP S (%)	GEN S (%)	MER S (%)	TAZ S (%)	VANC S (%)	AMP S (%)	ERY S (%)	CLIND S (%)
E. coli	5(83)	2(33)	0(0)	2(33)	0(0)	3(50)	2(33)				
Klebsiella	10(100)	2(20)	1(10)	4(40)	5(50)	8(80)	3(30)				
Pseudomonas	3(100)	0(0)	1(33)	2(67)	2(67)	3(100)	3(100)				
Acinetobacter	7(100)	3(43)	1(14)	4(57)	5(71)	5(71)	1(14)				
S. aureus	1(17)	1(17)	0(0)	2(33)	2(33)	5(83)	2(33)	3(50)	1(17)	2(33)	2(33)
M. morganii	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)	0(0)				
Proteus v.	1(100)	1(100)	0(0)	0(0)	0(0)	1(100)	1(100)				

S – Sensitive, AMP – Ampicillin, GEN – Gentamycin, CEF – Ceftriaxone, AMO – Amoxclavulinic acid, CLIN – Clindamycin, MER – Meropenem, TAZ – Piperacillin Tazobactam, ERY – Erythromycin, CIP – Ciprofloxacin, AMK – Amikacin

Factors associated with a positive swab culture among neonates with spina bifida

The likelihood of isolating bacteria from a spina bifida lesions was significantly increased by presence of bladder-bowel incontinence (p=0.025).

Table 5: Factors associated with having a positive swab culture among neonates with spina bifida at MNH.

Factors	**Swab culture bacteria growth**		**Total**	**Fisher's exact**
	Yes	**No**	**n=47**	**p-value***
Site of the defect				
Thoracolumbar	7(18%)	2(29%)	9	
Lumbar	18(45%)	3(43%)	21	5.306
Lumbosacral	12(30%)	1(14%)	13	
Sacral	3(7%)	1(14%)	4	0.447
Appearance of the lesion				
Cystic ruptured	17(42%)	4(57%)	21	
Cystic intact	22(55%)	3(43%)	25	5.724
Big ulcer	1(3%)	0(0%)	1	0.295
Size of the lesions				
1-3cm	0(0%)	0(0%)	0	6.191
>3-5cm	26(65%)	5(71%)	31	
>5cm	14(35%)	2(29%)	16	0.215
Condition of the lesion				
Clear CSF leakage	4(10%)	0(0%)	4	
Pus discharge	6(15%)	0(0%)	6	
Necrotic tissues	24(60%)	6(86%)	30	9.192
clean	6(15%)	2(4%)	8	0.082
Urine/fecal incontinence				
Yes	38(95%)	5(71)	43	7.241
No	2(5%)	2(29%)	4	**0.025**

***Fisher's exact test was used*

Discussion

The study aimed to describe the clinical profile, to determine common bacterial isolates colonising neonates with spina bifida and antibiotics susceptibility pattern at Muhimbili National Hospital. There was minimal prenatal diagnosis of spina bifida (9.1%), which commonly presented with open cystic lesions at the lumbar spine. Majority (85.1%) of the spina bifida lesions were infected with a predominantly gram negative bacteria (Klebsiella *spp*) which were found to be resistant to the first and second line antibiotics stipulated in our neonatal care guideline. The likelihood of isolating bacteria from the spina bifida lesions was significantly higher in neonates presenting with bladder-bowel incontinence.

Diagnosis of Spina bifida

Prenatal diagnosis of spina bifida is now possible using obstetric ultrasound which is cheaper, non-invasive and widely accessible in most part of the world. Although almost two thirds of our participants reported to have undergone a prenatal USS examination, only 9.1% of the neonates were diagnosed with SB prenatally. This is alarmingly low and contributes to delays in early intervention including counseling and neurosurgical repair. Similar findings have been reported in studies done in other LMICs such as Cameroon (2008) and Iran (2006) which showed only 11.6% and 24.3% of neonates having prenatal diagnosis of spina bifida respectively (5, 6). These findings can be due to lack of knowledge on the ultrasound markers for SB among the sonographers performing obstetric USS or that prenatal screening for major congenital anomalies has not been adequately advocated.

Size and location of spina bifida

The severity of complications of spina bifida has been associated with the size of the defect and its location along the spine. In this study, majority of the participants had a medium to large sized lesion with more than one third located on the lumbar spine. This is similar to studies done in Zambia (2018) and Turkey (2013) which reported the lumbar spine as the common locality for SB (9, 10). However, other studies have reported the lumbosacral region to be the most common site for SB (11, 12). The level of the SB is an important factor in determining the neurological status and functionality; and might be indirectly contributory to the risk of bacterial colonisation, if associated with bladder-bowel incontinence.

Mode of presentation of spina bifida

All the participants presented with open spina bifida lesions of which 40% had ruptured sacs with CSF leakage. This may probably be contributed by the mode of delivery which was mainly by SVD and may be a source of birth trauma due to a protruding cystic lesion on the back. This correlates with findings from a study done in Ivory Cost in 1997 which found that babies delivered per-vagina had a high risk of ruptured spina bifida lesions compared to those delivered by caesarian section (13). In this study, almost two thirds of the participants had necrotic tissues on their spinal bifida lesion, including those with intact sacs whilst a further 11.5% had pus discharging lesions. The necrotic tissues have been related to the effect of the amniotic fluid on the spina bifida lesions leading to unregulated degeneration of the cells (14).

Complications associated with spina bifida

In this study, majority of the participant had associated complications including: congenital hydrocephalus, paraplegia and bladder-bowel incontinence whilst close to one fifth had talipes equinovarus. The findings were much higher compared to those in a study done in Cameroon in 2017 where 61% of their participants had bladder and bowel incontinence while 65.3% had lower limb motor and sensory deficits and 65.3% had hydrocephalus respectively (11). The differences could be explained by the fact that in their study 17.5% had meningocele while most of the participants in our study had myelomeningocele the latter of which has spinal cord involvement and is likely to be associated with infection, hydrocephalus, double incontinence, motor dysfunction to the lower limbs and talipes equinovarus.

Common bacteria isolates and susceptibility pattern from spina bifida defects amongst neonates at MNH

According to the Mitali *et al*, all open spina bifida defects should be repaired within 48hours post-delivery, to mitigate the risk of bacterial infection (2). In this study, less than half of the participants were admitted within 48 hours post-delivery but none of them underwent surgical repair within that time. Hence the proportion of infected spina bifida lesions was found to be very high (85.1%). The delay in surgical closure of open SB could be explained by the lack of a SOP and proper multidisciplinary coordination of care of neonates with SB in our set up.

The predominant bacteria colonizing SB defects in this study were gram negative bacilli consisting of *Klebsiella species* of which one isolate was found to be extended spectrum beta lactamase

(ESBL) releasing *Klebsiella spp*. On the other hand the main gram positive bacteria found to colonize SB lesions was *Staphylococcus aureus* of which one isolate was coagulase-negative *S. aureus* (CONS). These findings are comparable to a study done in Brazil (2017) where the most common bacteria isolated was *Klebsiella pneumonia* (15). This was contrary to a study done in Sudan (2018) where most the common bacteria isolated was *Staphylococcus aureus* (16).

Neonates with open spina bifida are started on antibiotics empirically until a laboratory confirmation of infection can be made. At MNH, the first and second line antibiotics commonly initiated are ampicillin-cloxacillin/gentamycin or ceftriaxone alongside local wound care of the lesion. Due to the high levels of resistance to first and second line antibiotics in our setting, as reported in the Lancet (2013) (8), it has been shown that these antibiotics may not cover hospital acquired bacteria isolated from SB lesions. This was exemplified in this study by the high levels of resistance (67%-100%) to most antibiotics except meropenem and amikacin shown by *Klebsiella spp.* Similarly S. *aureus* showed a high susceptibility to meropenem, a moderate sensitivity to vancomycin and high resistance to most other antibiotics. This findings were similar to those found in the study done in Sudan (2018) where gram positive bacteria were resistant to methicillin but susceptible to vancomycin, while gram negative bacteria were resistant to cephalosporins but sensitive to imipenem (16). In the Brazilian study done in 2017, there were slightly different findings whereby *Klebsiella spp* was sensitive to ciprofloxacin and meropenem and resistant to amikacin and gentamicin (15). Apart from geographical differences, over prescription of gentamycin as a first line antibiotic in our setting may have contributed to the emerging resistance. On the other hand, amikacin is reserved as a second line drug for multidrug resistance tuberculosis (MDR TB) and therefore has been minimally prescribed and which might explain the low resistance in our setting.

Factors associated with having a positive swab culture among neonates with spina bifida at MNH

There was a significantly increased likelihood of isolating bacteria from the spina defects of the neonates who had coexisting bladder-bowel incontinence. This can be explained by the fact that majority of the lesions had necrotic tissue which served as a nidus for bacterial colonisation, whilst double incontinence increased the risk of ascending infection from faecal contents. However, there is paucity of published data for comparison of this findings.

Conclusion

Majority of the participants had open, medium to large lesions located in the lumbar area, 40% of which had ruptured resulting in a very high bacterial colonisation rate of >85%. The very low threshold (9.1%) for prenatal diagnosis of spina bifida by obstetric ultrasound may have contributed to lack of prior planning for immediate surgical repair within 48hours of birth, posing a high risk for infection. Most spina bifida defects were colonised by gram negative bacteria commonly *Klebsiella spp* sensitive to meropenem and amikacin, but resistant to the first and second-line antibiotics recommended in our national guidelines. Neonates who had bladder-bowel incontinence were more likely to have bacteria colonisation of their spina bifida lesions.

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