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Antibiotics Susceptibilities of Isolates from Eye Swabs of Babies Delivered through Spontaneous Vaginal and Caesarean Section Deliveries.

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Abstract

Purpose: The study aims to investigate the antibiotics susceptibilities of isolates from eye swabs of babies delivered through Spontaneous Vaginal (SVD) and non- elective Caesarean Section (CS).

Materials and Methods: This was a three-month prospective cross-sectional study, with samples as eye swabs of new born babies delivered in the maternity ward of Aminu Kano Teaching Hospital. Samples were collected not more than 30 minutes after delivery from 82 neonates (50 SVD and 32 CS) and stored in the refrigerator. It was evacuated in ice pack containers in batches within 24 hours to the microbiology laboratory for culturing, characterisation and sensitivity.

Results: The result showed that E. coli was isolated in 25(30.5%) of the samples, *P. aeruginosa*, 22(26.8%), S. aureus, 21(25.6%) and *K. pneumoniae* 14(17.1%). In the SVD group, E. coli was isolated in 16(32%) of samples, *P. aeruginosa*, 14(28%), S. aureus, 11(22%) and *K. pneumonia*, 9(18%). In the CS group, *S. aureus* was isolated in 10(31.3%) samples, *E. coli*, 9(28.1%), *P. aeruginosa*, 8(25.0%) and *K. pneumonia*, 5(15.6). There were 47(77%) SVD and 14(23%) CS samples that were resistant to three (3) or more antibiotics but this difference was not statistically significant (p=0.157). Babies born through SVD had higher bacterial isolates when compared with those delivered through CS, but this was also not statistically significant (p=0.787).

Conclusion: The mode of delivery had non-significant association with bacterial isolates from eye swabs of new born babies. We recommend Randomized Controlled Trial to establish the risk of bacterial isolates in new born babies' eyes.

Keywords: Spontaneous Vaginal Delivery, Caesarian Section, Isolates, Antibiotics susceptibilities..

Introduction

Neonatal eye infection occurs in babies exposed to infection during delivery, particularly during passage through an infected birth canal;[1,2,3,4] even in spontaneous Vaginal Delivery (SVD) that is without the use of tools like forceps or vacuum to bring out the baby. In-term babies, pre-mature rupture with a prolonged time before the onset of labour enhances the risk of neonatal eye infection because it usually leads to frequent vaginal examination (especially digital examination) which may contaminate the foetus exit parts with microbial agent[5,6,7,8,9,10,11]. In all pregnancies, premature rupture of membrane (PROM) may be seen in about 10.7%[5,6].

Pre-term or low birth weight new born babies are at higher risk of infection, especially when there is incidence of PROM during labour[10,11]. Newborn eye infection may be due to gram positive bacteria or coagulase positive *Staphylococcus*[12]. Other bacterial pathogens that can inhabit neonate conjunctiva at birth include *Streptococcus pyrogenes*, *Pseudomonas aeruginosa*, *Viridians streptococcus*[12].

In Caeserean Section delivery (CS)[13,14,15,16], babies do not come in contact with the natural birth canal. However, it was reported[16] that growth yields of *Staphylococcus aureus, Corynebacterium and Propioni bacterium acnea* were higher in CS when compared to SVD. These may be primarily acquired by the presence of bacteria in the surrounding air and also the level of care given by the hospital personnel[17,18].

The microorganisms in the maternal birth canal usually affects the flora in the early neonatal life of infants born through Spontaneous Vaginal Delivery (SVD)[18,19]. Thus, by contact with the vaginal secretions and the saprophyte and/or disease-causing bacteria present in the mother's birth canal, neonatal conjunctival bacterial community appears or neonatal conjunctivitis develops[20].

Bacterial acquisition of resistance to antibiotics is a challenge to the effective treatment of neonatal infections[19], hence the need for understanding of the distribution of the conjunctival bacterial flora with the mode of delivery. A significant number of disorders arising from bacterial infections can affect the eye of a new born baby and these may cause a serious repercussion on child's health if not properly treated[20]. The study was aimed at comparing the antibiotics

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susceptibilities of isolates from eye swabs of babies delivered through spontaneous vaginal and caesarean section.

Materials and Methods

This was a hospital based 3 months cross sectional observational study carried out between March and May, 2021. The sample collections were at labour ward and maternity theatre of Aminu Kano Teaching Hospital, Kano, while the laboratory analysis was done at the department of Microbiology, Bayero University, Kano. The study involved 82 neonates, of which 50 were delivered through SVD and 32 through CS. The samples from one eye of the new born babies who met the inclusion criteria were collected within 30 minutes of birth, by gently rolling a sterile swab stick along the conjunctival membrane of the babies while wearing a sterile hand glove. The swab stick containers were numbered and labelled serially as collected for easy identification. All samples collected were stored in the refrigerator and evacuated in ice pack containers in batches within 24 hours to the microbiology laboratory for culturing, characterisation and sensitivity profiling.

All new born babies who met the inclusion criteria within the period of the study were included in the study. The Inclusion criteria includes babies who were delivered in AKTH only, who had no ocular morbidity nor received any ocular prophylaxis at birth and whose parents gave consent.

Processing of samples

Swabs were anaerobically cultured in sterile Blood agar, MacConkey agar, Nutrient agar Mannitol salt agar and Muller-Hintol agar at 37^0C for 24hours. Isolates were identified -culturally, morphologically, biochemically and by sugar fermentation according to standard protocols[21,22]. Antibiotics susceptibilities of the isolates were determined using the disc-diffusion method. The result of identification, characterization and sensitivity profiling for all the bacterial isolated were observed.

Data Analysis:

Data was analyzed using SPSS version 22.0(SPSS Inc. Chicago, IL, USA). Basic descriptive statistics were performed on collected data using charts, frequencies and percentages. Fisher's exact test was used to determine if there was any relationship between mode of delivery and bacterial growth isolates (yield). The Significance level was set at $P < 0.05$.

Ethical Approval

Ethical approval was obtained from the research ethics committee of Aminu Kano Teaching Hospital, with Ref. NHREC/21/08/2008/AKTH/EC/2786 and dated, 17th February, 2020. The study adhered to the tenets of Helsinki Declaration.

Results

Antibiotic susceptibility test of isolates from eye swabs from conjunctiva of a total of eighty-two eyes (n=82) of neonates comprising 50(61%) delivered through spontaneous vaginal delivery (SVD), and 32(39%) delivered through caesarian section (mean age 20 ± 10.52 minutes) was carried out in this study. The result of the identification of organisms isolated is presented in table 1. Four distinct bacteria were identified from all the samples

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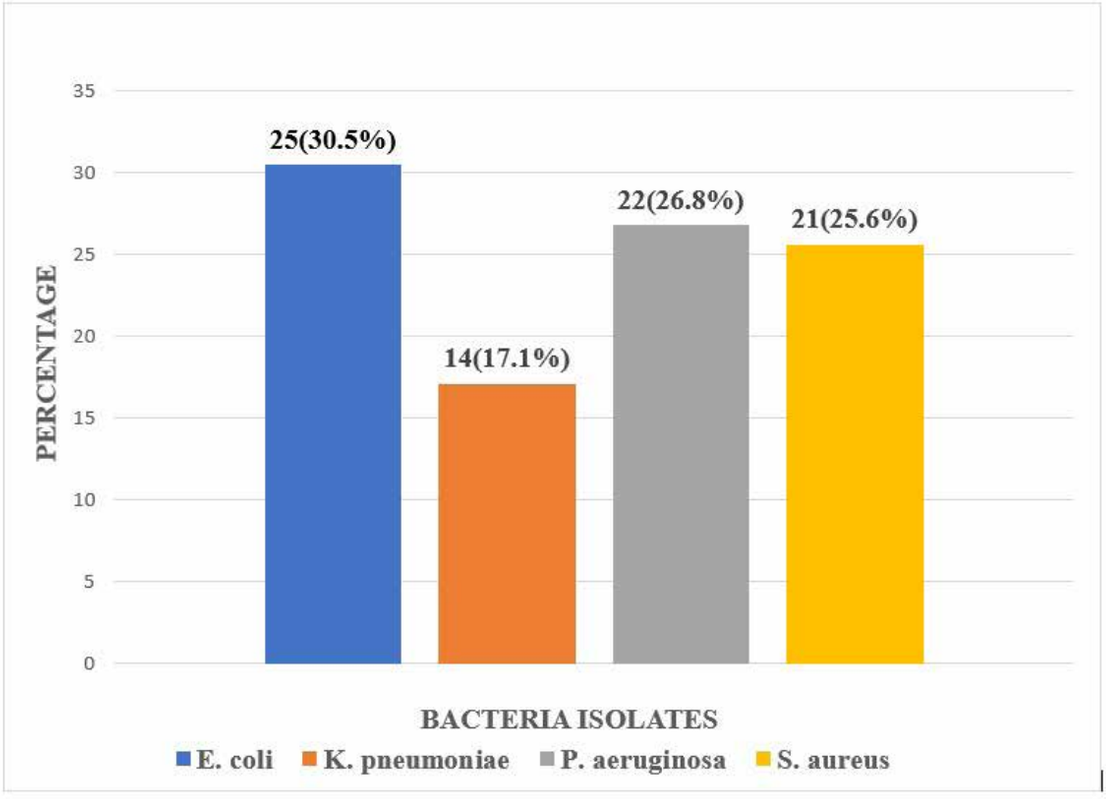
Table 1: Bacterial isolates identified from the samples

S/N	Colonial appearance after incubation	Morphological characteristics	Bacterial species
1	Colonies appeared as circular, low convex, entirely opaque, shiny and butyrous, ranging from creamy white to deep golden colour grown in nutrient agar plate after 24hrs incubation at 37^{o}C.	They are uniformly gram-positive (+ve) cocci, arranged in irregular, grape-like clusters. They are also in pairs and some in singular cells	*Staphylococcus aureus*
2	They appeared as pinkish, metallic and golden, shiny colonies on eosin methylene blue agar plate after 24hrs incubation at 37^{o}C	They are uniformly gram-negative (-ve) short and plump-rods cells in appearance	*Escherichia coli*
3	Light pink, viscid, mucoid and swarming colonies appears on McConkey agar plate after 24hrs incubation at 37^{o}C	Short and fairly long bacilli. Produced a capsules and non-motile cells, stained uniformly gram-negative (-ve) bacilli	*Klebsiella pneumonia*
4	The colonies appear greyish in colour Grown aerobically at 37^{o}C with opaque smooth transparent appearance on nutrient agar plate after 24hrs incubation	They are uniformly gram-negative (-ve) rod-shaped, non-sporing	*Pseudomonas aeruginosa*

Biochemical characterization tests were performed to confirm at specie level the bacterial isolates. Four distinct bacterial species were isolated as shown in table 2.

Table 2. Biochemical characterization test for confirmation of the bacteria isolated at specie level.

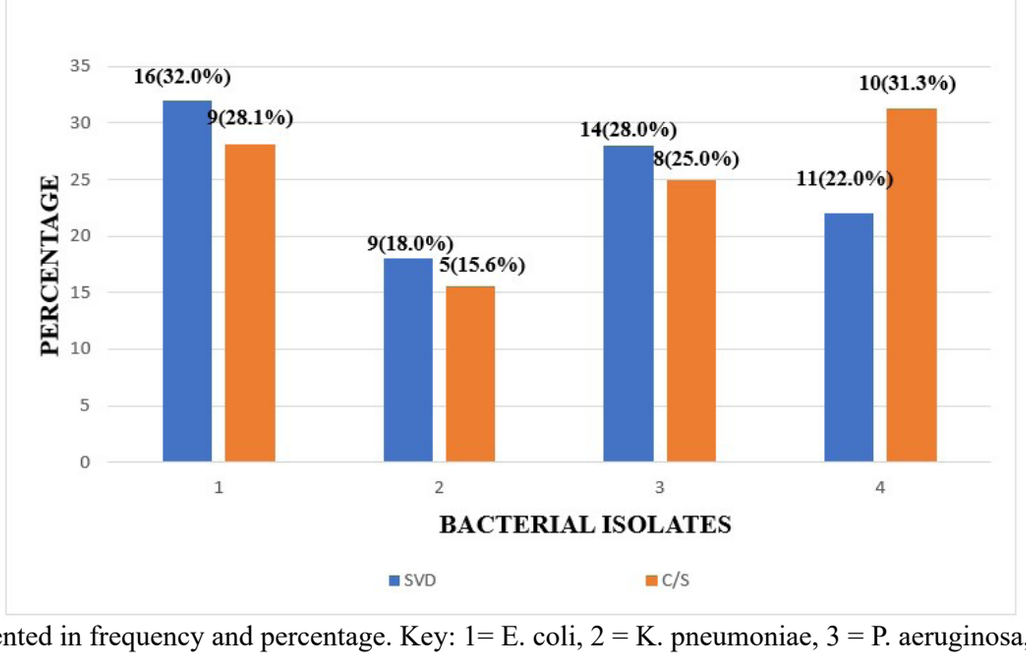
S/N	Tests	Bacterial Isolates	Remarks
1	Catalase Test	Staphylococcus aureus	+
	Coagulase Test		+
	Mannitol Test		+
	Glucose Test		+
2	Indole Test	Escherichia coli	+
	Methyl red Test		+
	Voges Proskaver Test		-
	Citrate Utilization Test		-
3	Mannitol Test	Klebsiella pneumonia	+
	Indole Test		-
	Methyl red Test		-
	Voges Proskaver Test		+
	Citrate Utilization Test		+
4	Cytochrome Oxidase Test	Pseudomonas aeruginosa	+



Data presented in percentage and frequency.

Figure 1. Distribution of bacterial growth yield from all the samples

The Distribution of bacterial isolates from all the samples showed that *E. coli* was the predominant organism with the highest percentage 25 (30.5%) of prevalence among the samples while *K. pneumonia* was the least 14 (17.1%) of the samples as shown in Figure 1.



Data presented in frequency and percentage. Key: 1= E. coli, 2 = K. pneumoniae, 3 = P. aeruginosa, 4 = S. aureus.

Figure 2: Bacterial Growth Yield by Mode of Delivery.

Figure 2 showed that *E. coli* and *S. aureus* were mostly isolated in babies delivered through SVD and C/S respectively, while *K. pneumonia* was the least isolated in both mode of deliveries. However, comparing the isolates among the SVD, *P. aeruginosa* 14 (28.0%) was the second most isolated bacteria followed by *S. aureus* 11 (22.0%). While among the C/S, *E. coli* was the most second isolated organisms followed by *P. aeruginosa* 8 (25.0%).

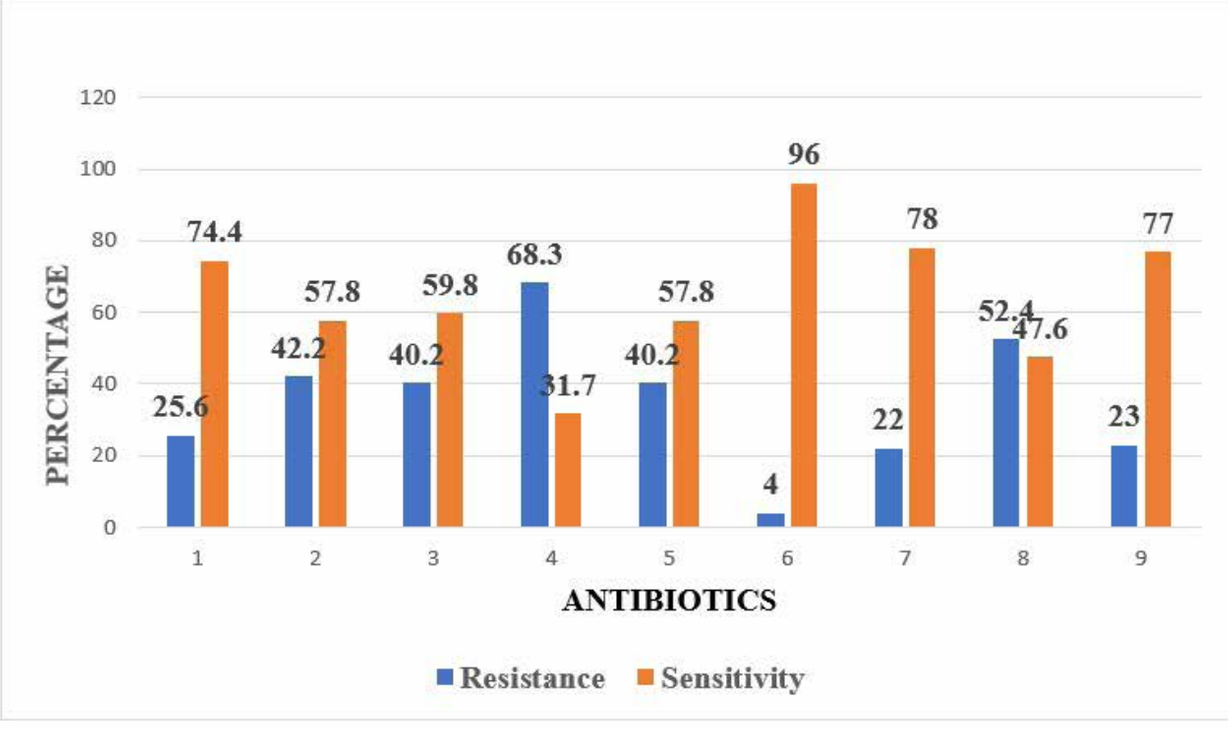


Figure 3: The Distribution of Antibiotics Susceptibilities of the isolated Organisms. Key: 1= Gen- Gentamycin, **2**= Aug- Augmentin, **3**= Cxm- Cefixime, **4**= Caz- Ceftaxidime, **5**= Cxr- Cefuroxime, **6**= Ofl- Ofloxacin, **7**= Cpl- Ciprofloxacin, **8**= Amp- Ampicillin, **9**= Nit- Nitrofurantoin.

The antibiotic resistance and sensitivity of various organisms isolated indicated that most of the isolates were sensitive to Ofloxacin (96%) followed with Ciprofloxacin (78%) while resistance occurred mostly with ceftaxidime (68.3%) and followed by Ampicillin (52.4%) across both modes of deliveries as shown in Figure 4.

Table 3: Relationship of Isolates Resistance to various antibiotics between SVD and CS groups.

Profile	SVD n (%)	C/S n (%)	Total n (%)	chi-square	p-value
Resistant to less than 3 antibiotics	3 (14.3)	18 (85.7)	21 (100)	2.000	0.157
Resistant to 3 or more antibiotics	47 (77)	14 (23)	61 (100)		

The frequency and percentage of isolates resistance to various antibiotics from samples collected from babies born through SVD showed that 47 (77%) samples were resistant to three (3) or more antibiotics, while 14 (23%) were observed in CS group as indicated in table (3). Pearson chi square showed that there was no relationship between the resistance of isolates from SVD and CS samples to antibiotics and were statistically insignificant (p=0.157).

Table 4: Frequency of antibiotic sensitivity against different isolates

S/N	Isolate	OFL	NIT	CPL	GEN	AUG	CXM	AMP	CXR	CAZ
1	Staphylococcus aureus	19	15	14	14	9	5	7	3	6
2	Pseudomonas aeruginosa	8	9	17	17	9	5	6	7	4
3	Escherichia coli	25	10	14	8	9	5	4	1	6
4	Klebsiella pneumoniae	17	13	12	11	9	7	3	2	8

Key: OFL- Ofloxacin, NIT- Nitrofurantoin- CPL- Ciprofloxacin, GEN- Gentamycin, AUG- Augmentin, CXM- Cefixime, AMP, Ampicillin, CXR- Cefuroxime, CAZ – Ceftazidime.

Table 4 shows the distribution of bacteria isolates sensitivities against different antibiotics. *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* were more sensitive to ofloxacin and ciprofloxacin as compared with Cefuroxime, Ceftazidime and Ampicillin respectively.

Table 5: The Relationship between Mode of Delivery and Bacterial growth yield.

Bacterial isolates	SVD n (%)	C/S n (%)	Test statistic	P – Value
E coli	16 (64.0)	9 (36.0)	3.067	0.787
K pneumonia	9 (64.3)	5 (35.7)		
P. aeruginosa	14 (63.6)	8 (36.4)		
S. aureus	11 (52.4)	10 (47.6)		
Total	50(100)	32(100)		

Based on the findings from the Fishers exact test, there was no relationship between Mode of Delivery (MOD) and bacterial growth yield and they were statistically insignificant (p=0.787).

Discussion

The study compared the antibiotic susceptibilities of isolates from swabs of conjunctiva of babies delivered through Spontaneous Vaginal delivery and Caesarean Section. It was observed that all the 82 samples were culture positive and that 61 isolates were resistant to 3 or more antibiotics. The fact that all the samples yielded bacterial growth is remarkable. From the total sample, *E. coli* was mostly isolated while *K. pneumoniae* is the least. Most of the isolates were sensitive to the antibiotic ofloxacin while resistance occurred mostly with ceftaxidime.

The fact that all samples collected yielded bacterial growth may confirm the suggestions that maternal factors like ingestion of adulterated foods just before labour and child birth; procedural factors such as cervical cerclage and amniocentesis; infant factors like pre-mature birth, low APGAR score, as well as prolonged labour, premature rupture of membrane, presence of bacteria in the environment in the labour

room as well as the level of care provided by the hospital staff may be predisposing factors[23]. The microbial flora of babies delivered through SVD and those of C/S does not differ as both mode of deliveries yielded same growths. However, *E. coli* was mostly isolated from the SVD group, while *S. aureus* was the most common isolate among samples in the CS group. Studies have shown that microbial community of the mother's vagina usually affects conjunctival flora in the earliest stage of life in neonates born through SVD[18,19]. This is because the baby comes into contact with vaginal secretions and saprophyte or pathogen bacteria present in the birth canal, leading to the emergence of newborn conjunctival flora which may lead to development of neonatal conjunctivitis. This may explain the higher growth yielded among these babies.

On the other hand, in babies born through CS, the new born do not come into contact with the birth canal. A study of babies born through CS showed that *S. aureus* was the most isolated organism perhaps due to their non-contact with the vagina. Colonization of the ocular conjunctiva in new born delivered through CS usually occurs within the first day of life[16]. *S. aureus* is a gram positive, non-motile, round shaped bacteria and is the most common cause of infection after injury or surgery. It is found in human skin, nose, groin, armpit contaminated water and it is transmitted through close contact with contaminated surfaces or sharing contaminated items. *S. aureus* is found in indoor and farm environments as a component to airborne dust, but the highest concentration is found in high hand touch areas such as door knobs, suggesting that human contacts in crowded areas such as in hospitals/academic institutions may play crucial role in *S. aureus* transmission via inanimate objects[17].This agrees with the findings in this study. Since the neonate born through CS does not contact the maternal birth canal, it means that these florae may be principally acquired through the presence of bacteria in the surrounding air as well as level of care given by the hospital personnel[17].

Another origin of neonatal infection is often through maternal gastro intestinal and genito-urinary tracts. This is because many of the mother's infections with these microbes do not disturb her[4]. *E. coli* is a rod-shaped bacterium of the family, Enterobacteriaceae. It is found in the environment, contaminated food and water, and lives harmlessly in the gastro-intestinal tract of healthy people. A particular strain of this organism is known to be pathogenic if it gets into the GIT by ingestion and later, passed out through the stool. Those mostly at risk of infection are newborn babies and pregnant women due to their low immunity. And because the mother's urethra lies close to the anus, the

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bacteria get easier access to the bladder where majority of urinary tract infections occur. This predisposes the mother to develop genito-urinary tract infection, which may be a source of infection in the eye of babies who were vaginally delivered[4]. This may also explain why *E. coli* was the most isolated organism from the test samples in this study. However, in a similar study[25], chlamydia trachomatis was the most isolated organism. The reason for the variation is not understood, though it may be due to the long duration of exposure the babies had in environment, since they collected the eye swab within 28days of birth and home discharge.

Prolonged labour is also another factor in neonates acquiring resident microbial flora. This is because prolonged labour leads to frequent digital vaginal examination which may contaminate the foetus exit parts with microbial agents thus leading to increased risk of infection[9]. Although the scope of this study excluded these variables, but existing literature helped to understand why and how these babies who participated in the study acquired their ocular microbial flora.

Premature rupture of amniotic membrane (PROM) which causes leakage of fluid before onset of labour is another factor that enhances babies acquiring resident microbiota. PROM is seen in about 10.7% of all pregnancies, 94% of the cases being found in mature fetuses while about 5% of them occur in immature fetuses weighing between 1,000gm to 2,500gm[5].

All neonates can develop infection, but preterm or low birth weights are at a particularly higher risk[12]. Although only two preterm baby deliveries were recorded during the study period, it was observed that samples from both yielded bacterial growths and was all resistant to three or more antibiotics. Owing to this low sample number, good comparison cannot be effectively made but it may be that antibiotic resistance seen in these two isolates could be as a result of poor maternal health or other factors like antibiotic abuse or misuse during pregnancy in order to prevent premature delivery.

Comparison of the relationship between mode of delivery and bacterial growth yield was statistically insignificant, meaning that no relationship exists between them. However, result from a similar study[24] says that a positive relationship exists as the authors observed that the presence of maternal factors4, prolonged labour[9] and preterm delivery[5] have a causal relationship with occurrence of neonatal conjunctivitis. Though other authors[16,25] did not make this conclusion in their studies, but their findings clearly showed that SVD babies always have higher bacterial growth yield or developed infection compared to the CS group. Another reason why it appears that the SVD group yielded more bacterial growth may be as a result of the disparity in sample size between the two groups as were also seen in the studies by other

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authors[16, 23, 25]. However, in another study[26] where the sample size from babies born through CS were higher than those of SVD, their findings showed greater number of them developed infection when compared with SVD babies.

Analysis of the antibiotic sensitivities of the bacterial samples collected from both groups showed that antibiotic resistance is greater in SVD group. Resistance to three or more antibiotics was greater in this group when compared with the CS group. This agrees with findings from a previous study[16]. This may be due to reasons mentioned above as proffered by those authors. Also, exposure of pathogens to anti-microbial agents is the main reason why resistance occurs. Genes responsible for resistance can mutate and then be transmitted asexually or by horizontal gene transfer, even in their biofilm state to the next microbial generation until they become the dominant population[27]. Since the disparity in population size of both groups is skewed in favour of the SVD group, together with events that may happen during the labour hours of the mother, it is possible that isolates from the SVD group may show more resistance compared to the CS group.

Most of the isolates from this study were highly sensitive to the antibiotic ofloxacin while resistant was greatest with ceftaxidime. *S. aureus*, *E. coli* and *K. pneumoniae* was more sensitive to ofloxacin while *P. aeruginosa* was more sensitive to ciprofloxacin and gentamicin. The reason for this is unknown. However, ofloxacin[28] and ciprofloxacin[29] are second-generation broad-spectrum fluoroquinolones while gentamycin[29] is a bactericidal aminoglycoside that is effective both against gram positive and gram-negative organisms but particularly useful for the treatment of severe gram-negative infections like *P. aeruginosa*[29]. Ceftaxidime is a third-generation cephalosporin beta lactam with a broad-spectrum antibiotic activity

Limitation and strength of the study

The scope of this study did not cover the period of ante natal activities of the mother of the babies. This would have helped to know the events that occurred prior to labour and delivery. Knowledge of this would have helped to ascertain indeed if there is a relationship between mode of delivery and bacterial growth yield. However, the time interval (30 minutes of birth) of our sample collection was an advantage of this over other studies

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CONCLUSION

This study shows that there was no relationship between mode of delivery and bacterial growth yield, though, babies born through SVD had more bacterial growth yield than the CS babies. Resistance to antibiotics were also most in babies born through SVD, and consequently, stand a higher risk of developing neonatal conjunctivitis than those born through CS. Therefore, greater effort toward improving the facilities and hygiene in the labour ward and maternity theatre room should be made by hospital managers. This could be in form of providing equipment like functional autoclaves, disinfectants and sterile hand gloves. These will help in breaking hospital transmission of infection.

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Conflicts of Interest

There is no conflict of interest.

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