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Prevalence of Ophthalmia Neonatorum in Kano State, Nigeria

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Abstract

Aim: This study aimed to determine the prevalence of Ophthalmia Neonatorum (ON) in Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria. **Methods:** This is a cross-sectional prospective study involving 53 neonates with ON born in AKTH from 1st February 2021 to 1st July 2021 and met the inclusion criteria. Eye swabs were taken from the Labor ward, Post-Natal and Special Care Baby Unit in AKTH, Kano. A data collection form was used to extract information about the participants including, age at presentation, gender, mode of delivery, severity of the condition and unit where the samples were collected. Chi-Square (X^2) was done using Statistical Product and Service Solutions (SPSS), version 25.**Results:** Out of 1862 live births, 53 had ON indicating a prevalence rate of 2.7%, 28(52.8%) and 25(47.2%) were males and females respectively. Fifty-three (53) samples of eye swabs were cultured from which *Staphylococcus aureus, streptococci species, pseudomonas aurignosa, Escherichia coli*, and *klebsellia* were isolated and only 3 yielded no growth. Three (3) to 5 days was found to be the highest age at presentation, 50(94.3%) of the cases were mild conditions, and 42(79.2%) were delivered through Spontaneous Vertex Delivery. No relationship existed between bacteria isolates, gender and location where the samples were collected with a p-value of 0.396 and 0.556 at 0.05 level of significance respectively. **Conclusion**: Babies presenting with ON were mostly in SCBU and Post-Natal. There is a need for regular eye swabs for microbiological investigations before treatment.

Keywords: Culture, Neonates*, Ophthalmia neonatorum,* Bacteria Isolates.

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Introduction

Ophthalmia neonatorum (conjunctivitis of the newborn) occurs within the first month of life. It is a bacterial or viral infection acquired during passage through an infected birth canal (Teoh & Reynolds, 2003). It commonly affects newborns usually within the first month of life. (Matejcek & Goldman, 2013) Ophthalmia neonatorum is inflammation of the conjunctiva caused by aseptic or septic factors. Aseptic conjunctivitis occurs when chemical irritants such as silver nitrate are instilled into the eye, while septic conjunctivitis is due to infections caused by bacteria or viral agents acquired from the genital tract of the infected mother (Teoh & Reynolds, 2003). Septic

conjunctivitis is responsible for 1.6-12% of all neonatal infections. (Teoh & Reynolds, 2003) Chlamydial neonatal conjunctivitis is caused by *C. trachomatis* serotypes D to K, which is the same as the serotypes that cause genital infections in men. (Jawetz, 2002) Chemical conjunctivitis secondary to silver nitrate solution application usually occurs on the 1st day of life, disappearing spontaneously within 2–4 days. (Darville, 2005) Meconium or other irritants have similar effects on the conjunctiva. Gonococcal neonatal conjunctivitis tends to occur 3–5 days after birth but can present later especially if topical prophylaxis has been used. (Darville, 2005) Chlamydia conjunctivitis usually has a late onset than gonococcal conjunctivitis. Incubation period of 5–14 days after delivery was reported with approximately 50% being bilateral. (Darville, 2005)

The treatment of *Ophthalmia neonatorum* has to be adequate because systemic complications and severe visual loss can occur particularly with *Chlamydia trachomatis* and *Neisseria gonorrhoea* which are leading causes of sexually transmitted diseases (Kolade, 2008). To avoid these complications, routine prophylaxis with antimicrobial eye drops or ointments is practised in many countries.

Given the changing etiological agents documented in Nigeria and other parts of the world as well as the evolving resistance of infective agents to routinely used therapeutic agents and the paucity of information on neonatal conjunctivitis in Nigeria. This present study was designed to determine the prevalence and identify the bacterial agents and ocular clinical presentation of neonatal conjunctivitis among newborn babies seen in AKTH. It will increase the scope of knowledge of eye care providers in the area of caring for patients with *Ophthalmia neonatorum*.

Materials and Method

This is a cross-sectional prospective study involving neonates born in Aminu Kano Teaching Hospital with *Ophthalmia neonatorum* for 6 months (1st February 2021 to 1st July 2021). The study involved 1862 (1027 males and 835 females) neonates, sixty- three (63) of the neonates had conjunctivitis, 10 out of them refused consent and 53 swabs were taken from the neonates with conjunctivitis whose parents gave consent. Aminu Kano Teaching Hospital is located in Kano State with coordinates 11.9666° N, 8.5639° E. The Hospital has its catchment areas as Kano, Jigawa, Bauchi, Katsina and Yobe States. The study involves 3 units in the hospital which include; The Special Care Baby Unit (SCBU), Post Natal and Labor Ward.

Ethical approval

Surrogate written consents were obtained from the parent(s) of the babies. Data on age, sex, bacteria isolated, unit where the samples were collected and mode of delivery were collected.

Ethical approval was obtained from the Health Research Ethics Committee of the Ministry of Health, Kano State (MOH/Off/797/T.I/2145). The purpose of this study was clearly explained to the parents of the participants before written informed consent was obtained. The instruments of data collection were the data collection form, registers of the three units and case folder.

Inclusion Criteria

To be eligible to be included in the study, participants met the following criteria:

1) Only neonates between 0-28 days who were born in Aminu Kano Teaching Hospital.

2) Those children whose parents or guardians consented to the study.

Method of Data Collection:
Sterile disposable swab sticks (silver healthcare), Culture plates and Culture media (Chocolate agar and Mac Conkey agar) were the materials used while the personal protection equipment included: Hand sanitisers, Disposable Hand gloves, Lab coat and Face mask.

Physical examination was conducted on all the study subjects and the severity of the conjunctivitis was scored in each case using the method described by Christian (1960). Scores were awarded as 1+, 2+ and 3+ for mild, moderate and severe cases respectively.

Specimen Collection
The patient was properly laid down on a bed at an appropriate height for the practitioner's and patient's safety and the head was well supported. Specimens were collected using sterile swabs. The patient's lower eyelid was dragged down to protect the cornea. Sample collection was by gently rolling a sterile swab stick along the eyelashes of the babies on either eye, bacterial swabbing was intended to collect free bacteria within discharge and on the tissue. Leeds (2013) suggested that obvious pus should be wiped off before swabbing takes place. The action of collecting this could be relatively gentle. Swabbing was done firmly along the lower fornix from the nasal side outwards; from the inner towards the outer canthus rotating the swab to collect any discharge. This sweeps organisms away from the lower punctum and picks up as many as possible. The swab was properly labelled and if swabbed from either of the eyes. Hands were properly washed at the beginning and end of the procedure and at any point where hands became contaminated. Bottles were labelled correctly after collection and stored before transportation to the lab for analysis.

The patient's parents/guardian was educated about the timescales for reporting. To let them know whether we would be in touch with them or not (for example if the organism was not found). Swabs were then transferred immediately to the Microbiology Department at Aminu Kano Teaching Hospital for Culturing. The culture media used were blood agar, chocolate agar and McConkey agar. The culture media were incubated at 37^0C aerobically for MacConkey agar plates while chocolate agar was incubated in 10% CO_2 atmosphere at 37^0C overnight. Gram Stain of the isolates from the culture was done to characterize and classify the isolated microbes. Presumptive isolates were stored on slants and biochemical tests were carried out on them for further characterization and identification.

Characterization and Identification of Bacteria Isolates
Bacterial isolates were characterized based on their colonial morphology as well as phenotypic (gram stain) and biochemical tests (oxidase, catalase, urease, citrate and sugar fermentation test) carried out as described by Cheesebrough (2005).

Morphological Characterization
Isolates were characterized morphologically by the use of characteristics such as texture, colour and shape.

Catalase test

Isolates identified as Gram-positive cocci were further differentiated based on their ability to split hydrogen peroxide into oxygen gas and water. The test procedure was described by Cheeesbrough[9]. In the test, a drop of water was added to a clean glass slide. A colony of the test bacterium was picked using a sterile wooden applicator, then emulsified in the water drop and hydrogen peroxide solution was added. An immediate bubbling indicated a positive catalase test. The catalase-positive Gram-positive cocci were then inoculated into mannitol salt agar as described by Kaiser, et al., (2012). Fifty-four grams of mannitol salt agar were dissolved in 1000ml of distilled water as recommended by the manufacturer. The mixture was sterilized by autoclaving at 121^0C for 15 minutes. The sterile medium was then dispensed in about 20ml amounts into sterile Petri dishes and after solidification, inoculation was carried out. A cooled flame-sterilized wire loop was used to pick an isolated colony of the test organism. This was streaked over a small area near the edge of the plate. The wire loop was again flame – sterilized and cooled, after which it was used to inoculate the remaining part of the plate by the streak method. The inoculated plates were then labelled and then incubated at 37^0C for 24 – 48 hours, after which they were examined for growth. Mannitol fermenting staphylococci produced yellowish colonies while non-mannitol fermenting staphylococci appeared pinkish or colourless.

Oxidase Test

The test was used to help in the identification of bacteria that produced the enzyme cytochrome oxidase, such as *Pseudomonas, Vibrio, Neisseria* etc. The bacteria were first of all separated into *Enterobacteriaceae* and non-*Enterobacteriaceae* using an oxidase test. *Enterobacteriaceae* are oxidase negative. The oxidase reagent was prepared by dissolving 0.1g of tetra methyl-p-phenylene-diamine dihydrochloride in 10 ml of distilled water. A filter paper was placed in a clean petridish and 2-3 drops of a freshly prepared oxidase reagent were added onto the filter paper. A sterile wooden applicator was then used to pick a colony of the test organisms which was emulsified onto the oxidase reagent – moistened filter paper. The development of a blue-purple colour within 10 seconds indicated a positive test. (Christian, 1960)

Citrate Utilization Test

Simmon's citrate medium was prepared according to the manufacturer's directions. Twenty four point two eight (24.28) grams of the powder were added into 1 liter of distilled water. The mixture was then heated to boiling point on a hot plate in order to completely dissolve the medium. The homogenous mixture was then dispensed in 5ml amounts into clean test tubes; the tubes were then capped with cotton wool and sterilized by autoclaving at 121^0C for 15 minutes. They were then kept in a slanting position to solidify. Inoculation of the medium was carried out as described by Cheesebrough (2005). A flame-sterilized wire loop was used to streak about ¾ of the medium with the suspension of the test bacterium. Incubation was done at 35^0C for 48 hours. A bright blue colour indicated a positive citrate utilization test. The absence of colour change in the medium indicated a negative test.

Sugar Fermentation Test

Triple sugar iron agar (TS1A) was prepared according to the manufacturer's directions by adding 65g of the

dehydrated powder into a flask containing 1 litre of distilled water. The flasks were heated to boiling point on a hot plate and the homogenous mixture was then dispensed in about 10ml amounts into clean test tubes. These were sterilized by autoclaving at 121°C for 15 minutes after which they were kept in a slanting position and then allowed to solidify. Inoculation of the medium was done as described by Cheesebrough (2005). A cooled, flame-sterilized wire loop was dipped into the suspension of the test bacterium. The loop was then used to streak the slope and stab the butt of the medium. The tubes were capped loosely and observed for colour changes of both slope and butt as well as gas and hydrogen sulphide production.

TSIA contains 1% lactose, 1% sucrose, 0.1% glucose, phenol red indicator, and FeSO4. The phenol red is yellow below pH of 6.80 and red above pH of 8.20. When only glucose is fermented, the acid produced is only enough to turn the butt yellow and the slant remains red. Thus, red slant, and yellow butt indicate glucose fermentation only. On the other hand, if either lactose or sucrose is fermented, a large amount of acid that turns both slant and butt yellow is produced. Thus, yellow slant and yellow butt indicated lactose and/or sucrose fermentation. If both the slant and the butt remain red, it indicated that no sugar has been fermented. The production of gas during fermentation is indicated by bubbles in the butt or cracking of the agar. Hydrogen sulphide production was indicated by black precipitate in the butt (Fankhauser, & Richard, 2005)

Growth on MacConkey Agar

MacConkey agar was prepared according to the manufacturer`s directions. The medium was dispensed in 15ml amounts into sterile plates. After solidification, inoculation was done by the streak plate method as described by Cheesebrough (2005). The plates were dried and a flame-sterilized wire loop was used to pick an inoculum from a saline suspension of the test bacterium. The inoculum was then spread onto a small area of the plate. The wire loop was sterilized and allowed to cool after which it was used to streak the inocula all over the plate, starting from the tail end of the prior inoculation on the same plate. The inoculated plates were labeled accordingly and incubated at 35ºC for 24-48 hours. Lactose fermenting colonies appeared pinkish on MacConkey agar while non–lactose–fermenting colonies appeared colourless (Kaiser, et al., 2012).

Growth on Mannitol Salt Agar

Mannitol salt agar was prepared according to the manufacturer's directions by dissolving the dehydrated powder in a litre of deionized water. The medium was sterilized by autoclaving at 121 ºC for 15 minutes. The medium was then dispensed aseptically in about 15ml amounts into sterile Petri dishes. The inoculation technique employed was exactly similar to that of MacConkey agar described above. The inoculated plates were incubated at 35ºC for 24 hours and examined for colonial appearance. The appearance of yellow colonies was considered as positive. (Cheesebrough, 2005)

Data Analysis

The data of 53 subjects were obtained and was presented using frequency, percentage and mean. These results were presented in charts and tables using Microsoft Excel 2016 version. The tabular

representation of data was chosen for easy understanding, interpretation and comparison of the findings obtained. Inferential statistics, correlation and Bi-variance tests for association were done using IBM SPSS (Statistical Product and Service Solutions) Version 25.

Result

A total of 53 (2.8%) neonates who had conjunctivitis participated in this study. The age range of participants in days, was 0-2, 3-5, 6-14 and >14, the minimum of age in days of the neonates was 2 while the maximum was 16. The mean age was 4.60 days with a standard deviation of 4.006. The mean age was 4.60 days with a standard deviation of
4.006.

Table 1: *Frequency Distribution of Age Group of the study Participants with Conjunctivitis.*

AGE GROUP (IN DAYS)	FREQUENCY	PERCENTAGE (%)
0-2	20	37.7
3-5	21	39.6
6-14	8	15.1
>14	4	7.5
TOTAL	53	100
GENDER		
Male	28	52.8
Female	25	47.2

A greater proportion of the participants [21 (39.6%)] fell within the age group of 3-5 days. There were 28 (52.8%) males and 25 (47.2%) females, giving a male-to-female ratio of 1.1:1. (table 1)

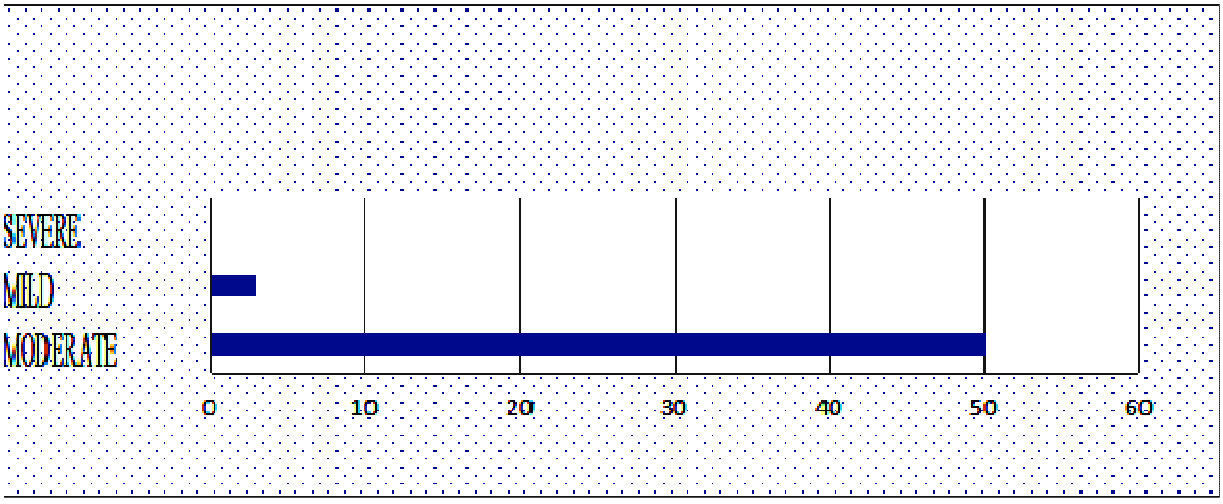

Figure 1: *Frequency of the Distribution of Severity of the Condition*

The severity of ON was also taken as mild, moderate and severe (Christian, 1960). The study revealed that 50(94.3%) mild conditions were the most prevalent with only 3(5.7%) moderate conditions while 0 (0%) severe condition was seen throughout the study. The

study revealed that mild conditions were the most prevalent with only 3 moderate conditions while no severe condition was seen throughout the study (figure 1).

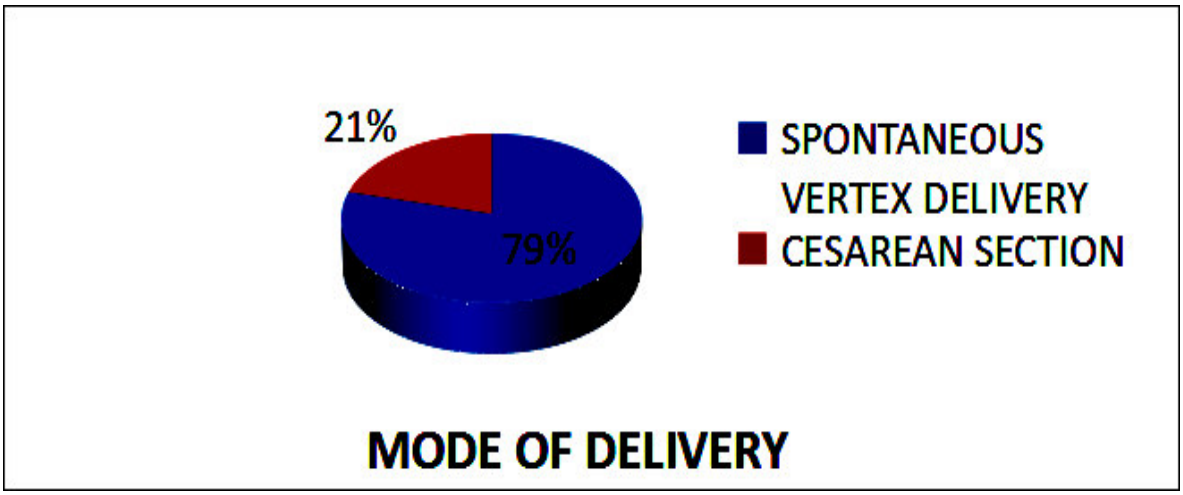

Figure 2: *Frequency Distribution of the Mode of Delivery of the Study Participants with Conjunctivitis.*

The modes of delivery of the participants were taken as SVD (Spontaneous Vertex Delivery) and CS (Caesarian Section). The modes of delivery of the participants were taken as SVD (Spontaneous Vertex Delivery) and CS (Caesarian Section). The SVD had the highest frequency of 42(79.2%) and the Caesarian section had the lowest prevalence of 11(20.8%). (Figure 2)

Table 2: *Distribution of Bacteria Isolated and Gender*

BACTERIA ISOLATES	MALE (n)	(%)	FEMALE (n)	(%)	TOTAL (n)	(%)
Streptococcus species	0	0	1	1.9	1	1.9
Psuedomonas aurisnosa	2	3.8	1	1.9	3	5.7
Klebsellia	1	1.9	1	1.9	2	3.8
Staphylococcus aureus	17	32.1	14	26.4	31	58.5
Escheridia coli	5	9.4	8	15.1	13	24.5
No Growth	3	5.7	0	0	3	5.7

The bacteria isolates revealed that *staphylococcus aureus* had the highest prevalence rate of 31(58.5%) and *streptococcus species* had only 1(1.9%) isolates. Three (3) out of the total swabs yielded no growth. In Table 2, the bacteria isolates against gender showed that males had the highest prevalence rate of 58.5% of staphylococcus, followed by *E.coli* which showed the second highest prevalence rate (24.5%). The result of the chi-square analysis output shows that the observed difference between bacteria isolates and gender was not statistically significant (χ^2= 0.396, p= 0.05).

Table 3: *Distribution of Bacteria Isolates by Unit where the Samples are Collected.*

BACTERIA ISOLATES	SCBU	(%)	POST NATAL WARD (%)		LABOR WARD (%)	
Streptococcus species	1	I.9	0	0	0	0
Psuedomonas aurisnosa	2	3.8	1	1.9	0	0
Klebsellia	2	3.8	0	0	0	0
Staphylococcus aureus	15	28.3	12	22.6	4	7.5
Escherichia coli	6	11.3	7	13.2	0	0
No Growth	3	5.7	0	0	0	0
Total	29	54.8	20	37.7	4	7.5

Cross tabulation of the location of the sample collected and bacteria isolates showed that 4(7.5%) samples collected at the labour ward were positive for *Staphylococcus aureus*, 12(22.6%) samples collected at the Post Natal ward were positive for *staphylococcus aureus*, 15(28.3%) samples collected at the SCBU were positive to *staphylococcus aureus* making a total of 31(58.5%) positive to *staphylococcus aureus*. (Table 3) However, this difference was not statistically significant (χ^2= 0.556, P = 0.05).

Discussion

The mean age of 4.6 ± 4.00 days at presentation in neonates with neonatal conjunctivitis in this study was lower than the mean age of 12.9 days documented in Zaria (Olatunji, 2004). The mean age of this study was exactly the same as 4.6 days recorded in Abakaliki (Ibekwe et al., 2007). The younger age at presentation in the present study may be due to the self-referral system of attending tertiary health centres as suggested by a study in Abakaliki (Ibekwe et al., 2007).

The male-to-female ratio of 1.1:1 in this study was the same as that reported by other researchers (Olatunji, 2004; Mohile, et al., 2002). It was, however not consistent with the studies in Abakiliki (Ibekwe et al., 2007), which reported a M: F ratio of 1.2:1. Males have an approximately 2-fold higher prevalence of infections than females, suggesting the possibility of a sex-linked factor in host susceptibility to infection (Gotoff, 2000). Male predominance with *Ophthalmia neonatorum* is also not consistent with findings documented in Tehran (Hossein et al., 2007) and Norway (Dannevig et al., 1992). However it is consistent with the findings from studies in India (Pandey et al., 1990) and Nairobi Kenya (Fransen et al., 1986) which showed male preponderance respectively. The male gender as a risk factor for increased neonatal infection has been attributed to Y-gene (Belyhun et al., 2018).

Staphylococcus aureus was the most commonly isolated bacteria in this study. This was in keeping with the finding from Zaria (Amoni, 1979) which reported *S. aureus* as the most common organism of neonatal conjunctivitis. It was also in keeping with the report in Benin City (Iyamu & Enabulele, 2003) and Abakalike (Ibekwe et al., 2007), where *S. aureus* was also the most prevalent organism. Studies from USA[24] (Jarvis, 1987) and UAE (Soltanzadeh, 2004) also showed *S. aureus* as the most common organism causing neonatal conjunctivitis. However, these results differed from a study carried out in Ilorin Nigeria (Kolade, 2008), which recorded *Chlamydia trachomatis* as

the predominant organism of neonatal conjunctivitis.

The presence of *S. aureus* in this study may suggest that most of the cases of neonatal conjunctivitis are post-natally acquired probably due to low levels of hygiene rather than during passage through the birth canal as suggested by a researcher in explaining the implication of *S. aureus* as aetiological agent of neonatal conjunctivitis (Vedantham, 2004). Low numbers of *streptococcus species* were found in this study. This was in contrast to the findings from Benin (Iyamu & Enabulele, 2003) and Ilorin (Kolade, 2008), where *streptococcus species* was not isolated. However, a study carried out in Zaria (Amoni, 1979) found *N. gonorrhoea* and *S. aureus* as the most implicated organisms of neonatal conjunctivitis. Two decades later, a study from the same centre showed a lower prevalence of *N. gonorrhoea* neonatal conjunctivitis. Similarly, a researcher from the same centre in Zaria did not isolate *N. gonorrhoea* in neonatal conjunctivitis (Abdulkadir, I.A. (2008).
These observations suggest reducing the importance of *N. gonorrhoea* as a cause of neonatal conjunctivitis. It may be suggested that the low rate of *N. gonorrhoea* isolation may be due to the availability of health facilities, improved health habits and improved antenatal care attendance according to a study in Kano (Mohammed et al., 2013).
The differences in isolated organisms from different centres may be a reflection of the socioeconomic status, personal hygiene of the individuals and predominant agents in the environment, which may differ from centre to centre. Laboratory factors, the fastidious nature of some of the organisms, the objective of the studies, locally available expertise and high ethical laboratory standards all may play a role (Abdulsalam et al., 2013) It may also be due to variations in the etiological agents of STIs and maternal genital flora in various centres. However, maternal genital flora was not examined in this study as it was not part of the objectives of the study.

The findings revealed that 2.7% of the neonates presented with ON in Aminu Kano Teaching Hospital, Nigeria, which was lower than the 5.4% recorded from three medical centres in Tehran (Hossein et al., 2007) and less than 19% observed among babies in rural areas of Northern Norway (Dannevig et al., 1992). In Benin, Nigeria (Iyamu & Enabulele, 2003), a study recorded 1.7% and 60.5% prevalence rates for gonococcal and staphylococcal infections respectively, which is consistent with the zero isolation of gonococcal and higher staphylococcal prevalence in this study. The observation in this study might be partly due to the bacteriological tests conducted before treatment. In addition, infants with this condition were mostly from parents of low socioeconomic status; accordingly, financial constraints, low literacy levels, and poor health-seeking behaviour might contribute to this condition. In a related study in Kano, it was found that low maternal education, lack of antenatal care attendance and low socio-economic status were significantly associated with positive bacteria isolates (Abdulsalam et al., 2013)

Poor antenatal care without early detection and treatment of vaginal infections or delivery in unhygienic environments by unskilled personnel are among the other risk factors for this condition, which is confirmed by the results obtained from Kaduna, Nigeria[28], but inconsistent with the findings from a

case-control study of neonatal conjunctivitis in Kaduna, Nigeria[13]. Olatunji (2004) observed that 49% of babies delivered in hospitals (vs. 34.5% delivered at home) developed ON. A study in Kenya, however, did not find birth in an unhygienic environment to be a significant risk factor (Adeyantso, 2004). We could not ascertain if any of the babies in this study became blind or had significant visual disabilities, although ON is known to cause blindness in approximately 10,000 babies annually worldwide.

The chi-square (X^2) analysis conducted on the relationship between bacteria isolates and gender of the participants and also between bacteria isolates and location of samples collected showed no statistically significant relationships between bacteria isolate by gender and bacteria isolates by a unit of the sample collected with a p-value of **0.396** and **0.556** respectively. This was consistent with a similar investigation by Belyhun and coworkers (Belyhun et al., 2018).

Conclusion

The findings revealed a prevalence of (50) 2.7% of *Ophthalmia neonatorum* in Aminu Kano Teaching Hospital, Kano, Nigeria; male neonates were mostly having ON at their early stage of life. However, most of the cases were mild and mostly delivered by Spontaneous Vaginal Delivery. It was also found that the condition was predominantly mild with no severe cases. *Staphylococcus aureus* and *Escherichia coli* were the commonest bacterial agents responsible for neonatal conjunctivitis in Aminu Kano Teaching Hospital, Kano, Nigeria. Babies presenting with *Ophthalmia neonatorum* were mostly in SCBU and Post-Natal and treatments for this condition were instituted without performing the relevant investigations.

Conflict of interest:
There is no conflict of interest.

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