Original Article

Antimicrobial Resistance Patterns Of Bacteria Isolated From Eye Swab Samples Of Newborn Babies At Tertiary Care Hospitals Of Karachi, Sindh, Pakistan

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Abstract

Objectives:

Antimicrobial resistance is one of the growing public health problems in developing countries like Pakistan. Therefore, this study aimed to determine the type of microorganisms acquired by newborns immediately after birth and the antimicrobial resistance patterns of the bacteria isolated from eye swab samples.

Methods:

Women were recruited from the Obstetrics ward of two tertiary care hospitals in Karachi, Pakistan. Immediately after birth conjunctival swabs were taken from one eye of the newborn. The information like mode of delivery, person conducted delivery, gestational age, and birth weight was noted from hospital records.

Results:

From 203 eye swabs collected, microbial growth was observed in 69 (34 % with 95 % confidence interval CI: 27.48% - 40.52%). Gram-positive bacteria were observed in 16 (23.2%) and Gram-negative bacteria were found in 50 (72.5%) and yeast was also seen in 3 (4.3%). The most common gram-positive organisms observed were Staphylococcus species in 16 swabs (20.3%) and E. coli in 20 (25.3%), followed by Acinetobacter species in 10 (12.7%), Enterobacter species in 8 (10.1%), Pseudomonas stutzeri in 6 (7.6%), Pseudomonas species in 6 (7.6%) and Pseudomonas aeruginosa in 4 (5.1%). All 4 (100%) Pseudomonas aeruginosa strains were resistant to Augmentin and Cotrimoxazole and from 16 Staphylococcus species, 87.7% were resistant to Penicillin, 56.3% to Erythromycin, 50% to Cloxacilinn, 43.7% to Tetracycline, 31.3% to each Cotrimoxazole, Fusidic acid, Gentamycin.

Conclusions:

The carriage of antimicrobial-resistant bacteria immediately after birth is high in the newborn of the lower middle-class population of Karachi. Raising awareness regarding the intake of antibiotics on prescription can also reduce the risk to the newborn baby and the mother.

Keywords Antimicrobial Resistance, Bacteria, Eye Swab, Newborn, Pakistan.

Introduction

Neonatal conjunctivitis is the inflammation of the membrane that lines the eyelids and covers the surface of the sclera. [1] Neonatal conjunctivitis is red-eye caused by irritation, a blocked tear duct, or infection in the first 28 days of life. [2] The neonates are more susceptible to eye infections because the newborn conjunctiva has lower levels of lysozyme and Ig A in tears and the flow of tears and tears film are not developed. [3] Several microorganisms can cause an eye infection. Bacteria that are most frequently associated with conjunctivitis in newborns are Staphylococcus aureus (S. aureus), Staphylococcus epidermidis, Klebsiella species, Escherichia coli (E. coli), Enterococcus species, Pseudomonas species and Corynebacterium species. [4-6]

Eye infections in neonates are one of the common problems requiring an antibiotic prescription by health care providers. [5] To prevent complications, antimicrobial eye drops or ointments are given as routine prophylaxis in many countries. [4-6] There is no routine eye prophylaxis is given to the newborn immediately after birth in Pakistan. [7] Very few studies reported the burden of eye infections during the neonatal period in Pakistan. [7] Furthermore, none of the earlier studies reported the type and antimicrobial resistance patterns of microorganisms carried by neonates immediately after birth. Antimicrobial resistance is one of the growing public health problems in developing countries like Pakistan. Therefore, we aimed to study the type of microorganisms acquired by newborns immediately after birth. Besides, we also determined antimicrobial resistance patterns of the bacteria isolated from eye swab samples of the newborn in tertiary care hospitals in Karachi, Pakistan.

Methods

A cross-sectional study was conducted in Karachi which is the most populous city in Pakistan. The study subjects were selected from the Obstetrics ward of two tertiary care hospitals in Karachi. These public sector hospitals serve predominantly low to lower-middle-class populations of the city. All women aged 15-49 years who had a singleton, vertex, vaginal delivery, or cesarean section were recruited. Women who had intrauterine death and with face or breech presentation, not willing to give written consent were excluded from the study. About 200 mother and baby pairs were recruited. The information like mode of delivery, person conducted delivery, gestational age, and birth weight was noted from records after seeking permission from the hospital authorities. Three midwives were recruited for data collection purposes in the obstetrics ward of the Hospital for the morning, evening, and night shifts. Data collectors were responsible for taking consent from the eligible women participant and filling out questionnaires. The eye swab of the newborn babies was taken by the medical doctors who were hired for the study purpose. A conjunctival swab was obtained from one eye of the newborn, before the application of any topical medications and immediately placed in AMIES Transport medium (Transwab Amies MW 170, Medical Wire and Equipment Co. Ltd., Corsham, England). The specific identification number was assigned to each specimen along with the date of specimen collection and transported to Dow Diagnostic Reference and Research laboratory, Karachi for culturing and identification of micro-organisms. All specimens were inoculated on fresh Blood agar, Chocolate agar, and Mac-Conkey's agar plate. The aerobic inoculated plates were incubated at 37 degrees Centigrade for 24-48 hours. All plates were examined for growth every day and negative plates were kept incubated for 7 days of initial inoculation. In the presence of growth on agar plates, pure cultures were made for each type of colony of bacteria. Final identification was made based on gram stain and results of biochemical tests. Antimicrobial susceptibility testing of bacterial isolates was performed by using the standard agar diffusion method on Mueller-Hinton agar (Oxoid, England). Antimicrobial resistance data were interpreted according to National Committee for Clinical Laboratory Standards (NCCLS).

Descriptive analysis was done through SPSS version 21.00 to generate mean, frequencies, and standard deviation for continuous variables, and proportions were calculated for categorical variables. Ethical approval was taken from the ethical review committee of Aga Khan University (AKU) before the implementation of the study. Permission for

conducting the study was also taken from hospital authorities. Informed and written consent was obtained from each participant before the interview and specimens were collected.

Results

Of the 205 mothers and newborns recruited, 203 neonatal conjunctival swabs were obtained. Two of the women refused to give eye swabs of their babies. About 147 (71.7%) of deliveries were conducted by medical doctors and 58 (28.3%) by a nurse or midwife. C-section was performed in 15 (7.3%) of recruited women and 114 (55.6%) of the mothers who gave birth to a male baby. The mean (± SD) gestational age of newborns was 38.5 (± 0.48) and the mean birth weight in kilograms was 2.94 (± 0.48) in newborn babies.

Of 203 neonatal conjunctival swabs taken from babies, microbial growth was observed in more than a third of the samples 69 (34 % with 95 % confidence interval CI: 27.48% - 40.52%). Two different types of bacteria were observed in 9 swab cultures and 3 different types of bacteria were noted in one swab culture result. Among positive swab cultures of the neonates' the number of colonies was in the category of few colonies in 50 (72.5%), moderate colonies in 15 (21.7%), and numerous colonies were found in 4 (5.8%) culture results. Gram-positive bacteria were observed in 16 (23.2%) and Gram-negative bacteria were found in 50 (72.5%) and yeast was also seen in 3 (4.3%).

Gram-positive bacteria include Staphylococcus species noted in 16 (20.3%) samples, Corynebacterium species in 2 (2.5%), and Micrococcus species in 1 (1.3%) samples. Gram-negative bacteria include E. coli in 20 (25.3%), Acinetobacter species in 10 (12.7%), Enterobacter species in 8 (10.1%), Pseudomonas stutzeri in 6 (7.6%), Pseudomonas species in 6 (7.6%), Pseudomonas aeruginosa (P. aeruginosa)in 4 (5.1%) and Klebsiella species in 3 (3.1%) samples. Yeast Candida albicans (C. albicans) were also observed in 3 (3.8%) of swab cultures (figure 1).

All gram-negative bacteria were sensitive to Amikacin, Imipenum, and Piperacillin/ Tazobactam. Among 20 E. coli bacteria isolated in eye swab cultures of newborn babies, 65% were resistant to Cotrimoxazole, 50% to Ampicillin, 20% to Cefixime, 10% to Ofloxacin and 5% to each Tobramycin and Gentamycin. All 4 (100%) Pseudomonas aeruginosa strains were resistant to Augmentin and Cotrimoxazole, followed by 50% to each Carbencillin, Cefotaxime, and Ceftriaxone (table 1). Among 3 types of gram-positive bacteria including Staphylococcus species, Micrococcus species, and Corynebacterium species all were sensitive to Amikacin and Vancomycin. Of 16 Staphylococcus species, 87.7% were resistant to Penicillin, 56.3% to Erythromycin, 50% to Cloxacilinn, 43.7% to Tetracycline, 31.3% to each Cotrimoxazole, Fusidic acid, Gentamycin. Furthermore, resistance to Chloramphenicol, Clindamycin, and Ofloxacin was also noted in 18.7% of Staphylococcus species (table 2).

Discussion

This study was carried out to determine the bacterial carriage in newborns immediately after birth and the antimicrobial resistance patterns of the isolated bacteria. We have observed the growth of microorganisms in 34% of eye swab samples of newborn babies. A previously conducted study in Islamabad, Pakistan reported only 29% positive cultures. [7] Generally, the reported prevalence is high in several previous studies conducted in other settings. [8,9] A study conducted in the NICU of a large university teaching hospital in Connecticut found 58% positive cultures of neonates. [8] An incidence of 84% from two hospitals in Argentina and Paraguay was reported earlier. [9] Similarly, a large study from Rajasthan, India, reported the presence of bacteria in 86 % of eyes examined. [10] The prevalence observed in our study is comparatively low.

We found gram-positive bacteria (Staphylococcus, Corynebacterium, and Micrococcus species) and gram-negative bacteria (E. coli, Enterobacter Klebsiella, and Pseudomonas species) in our study. The types of microorganisms identified in our study are consistent with other studies. The most common isolate was S. aureus in 65% and Klebsiella species in 23% in the previous study conducted in Pakistan. [7] They have also not observed any Neisseria gonorrhea

from swab samples. [7] The previous study conducted in Argentina and Paraguay also reported similar bacterial isolates. [9] The most common bacteria isolated were coagulase-negative Staphylococcus (CoNS) in 38%, Propionibacterium species in 20% and Corynebacterium species in 16%, and no Neisseria gonorrhoeae were isolated. [9] CoNS was also predominantly noticed in 75% of samples of neonates in Connecticut. [8] In a study conducted in the hospital setting in Ludhiana, India, the most common organism was S. aureus (35.2%) followed by Enterococcus species (4.3%), Pseudomonas species (3.7%), Klebsiella species (3.5%) and E. coli (2.8%).[5] CoNS, Diphtheroids, and Yeast were also identified as organisms causing conjunctivitis in newborn babies in India and we also identified these microorganisms in newborn babies' conjunctiva at birth. [5]

The most common gram-negative bacteria identified in our study was E. coli, which is commonly acquired from the mother during birth. E. coli, Enterobacter, and Klebsiella species belong to the Enterobacteriaceae family and are an important cause of urinary tract infection (UTI) in pregnant women. [11] Other microorganisms like Staphylococcus species and C. albicans are found as the normal flora of the female genital tract and are the cause of UTI in females. [11,12] In our study, the presence of these microorganisms in the conjunctiva of newborn babies at birth can be a result of contamination from the mother's vagina. Other studies have also reported that potentially pathogenic bacteria can be found in the conjunctiva of vaginally delivered infants which they acquire while passing through the birth canal of their mothers. [13, 14] It has been exemplified by many studies that neonatal conjunctivitis is often contracted while passing through maternal passage. [5, 15, 16] In a study by Rao et al, it is found that in 85% of mother and baby dyads, microorganisms from the vagina and conjunctiva were similar. [14]

Conjunctivitis is not always manifested in newborns even in the presence of conjunctival flora. [6] Studies have reported that if neonatal conjunctiva is contaminated at birth then half of the cases of neonatal conjunctivitis usually occur within the first 48 hours of life, 57% of the conjunctivitis cases appeared in 48-72 hours after birth and 92% of the conjunctivitis of bacterial origin, developed within the first week. [5, 6, 14] Most cases of pediatric conjunctivitis in developed countries are bacterial in origin and resolve without any vision loss.

The availability of antibiotics without prescription and irrational use of antibiotics has led to the development of resistance in bacteria to commonly used antibiotics. High resistance was noted in gram-positive and gram-negative bacteria identified in our study samples. In our study, 65% of E. coli were resistant to Cotrimoxazole, 50% to Ampicillin and 20% to Cefixime, 10%. All P. aeruginosa strains were resistant to Augmentin and Cotrimoxazole, followed by 50% Ceftriaxone. From Staphylococcus species 88% were resistant to Penicillin, 56% to Erythromycin, 44% to Tetracycline, 31% to each Cotrimoxazole and Gentamycin. Previous UK, Ethiopia, and Portugal studies also reported high resistance to these antibiotics. [17, 18, 19] The generalization of study findings is limited as we sampled newborn babies from two public sector hospitals, which is not representative of the community settings of Karachi. However, the study participants are not very different in their socioeconomic status and belonged to the lower and lower-middle-class of communities; therefore this study represents the situation of these communities.

To summarize, the carriage of antimicrobial-resistant bacteria immediately after birth is high in the newborn of a lower and lower middle class of the population. The prevalence of bacteria in eye swabs was high in our study, and most bacterial isolates were resistant to commonly used antibiotics. Therefore, health professionals should be made aware of this risk and before prescribing any antibiotic it should be essential to conduct a drug susceptibility test. Raising awareness regarding good personal hygiene and the intake of antibiotics on prescription can also reduce the risk to the newborn baby and the mother.

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Financial Disclosure

The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

Ethical Approval

Ethical approval has been obtained from the ethical review committee (ERC) of Aga Khan University Hospital, Karachi, Pakistan.

Authors' contributions

Conceptualization: JM, SS. Data curation: JM. Formal analysis: JM, SIA. Funding acquisition: JM, SS. Methodology: JM, SS, NFZ. Project administration: JM, SS, MRK. Visualization: JM. Writing - original draft: JM, SS. Writing - review & editing: JM, SS, NFZ, MRK, SIA. All authors reviewed and approved the manuscript.

Competing Interests

The author (s) declares that they have no competing interests.

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Figure 1. Type of microorganisms identified in eye swabs of newborn babies at birth

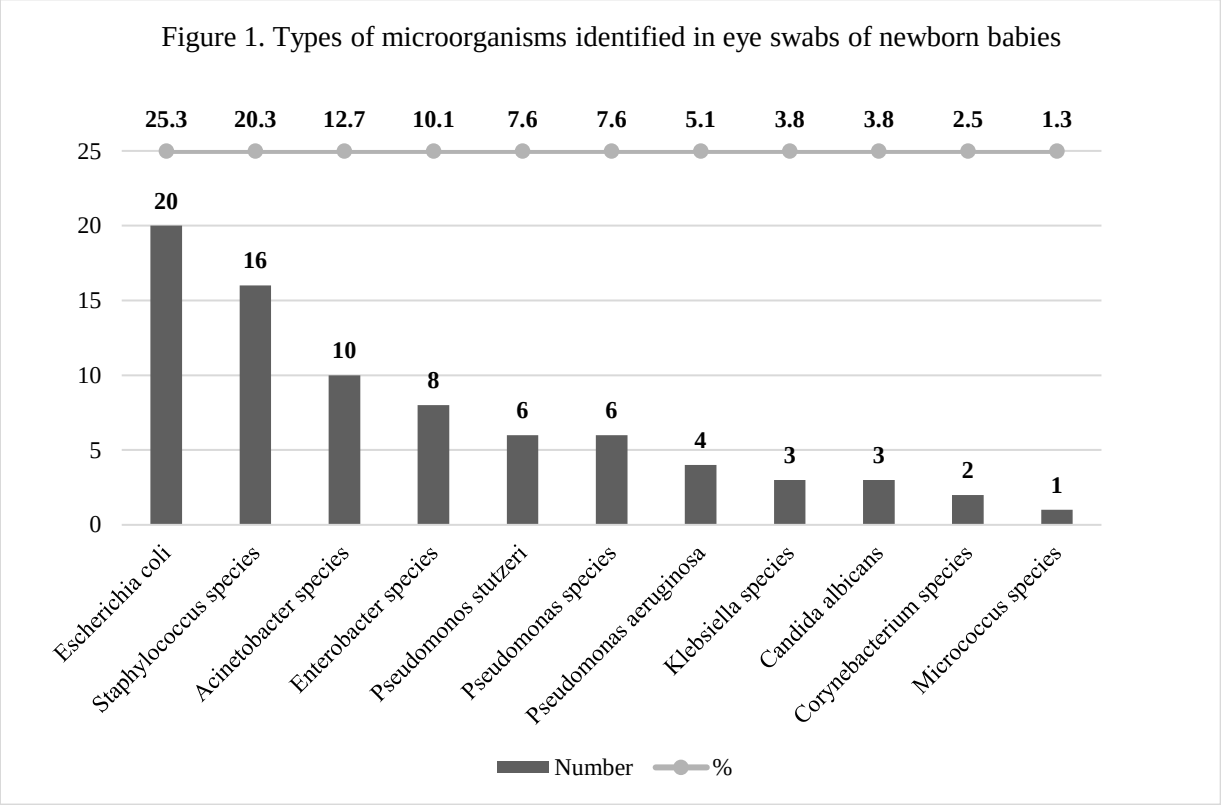

Table 1. Antimicrobial resistance patterns of gram-negative bacteria isolated in eye swab cultures of newborn babies

Gram negative sensitivity testing results	Escherichia coli (n=20)		Acinetobacter species (n=10)		Enterobacter species (n=8)		Klebsiella species (n=3)		Pseudomonos stutzeri (n=6)		Pseudomonas species (n=6)		Pseudomonas aeruginosa (n=4)	
	R %	S %	R %	S %	R %	S %	R %	S %	R %	S %	R %	S %	R %	S %
Amikacin	0	100	0	100	0	100	0	100	0	100	0	100	0	100
Ampicllin	50	50	60	40	87.5	12.5	100	0	25	75	100	0	25	75
Augmentin	10	90	20	80	25	75	0	100	0	100	33.3	66.7	100	0
Azactam (Aztreonam)	30	70	30	70	12.5	87.5	0	100	20	80	50	50	25	75
Cefixime	20	80	50	50	25	75	33.3	66.7	33.3	66.7	100	0	25	75
Carbencillin	0	100	10	90	12.5	87.5	0	100	50	50	33.3	66.7	50	50
Cefotaxime	15	85	20	80	12.5	87.5	0	100	0	100	40	60	50	50
Ceftriaxone	15	85	20	80	0	100	0	100	0	100	16.7	83.3	50	50
Cotrimoxazole	65	35	50	50	12.5	87.5	0	100	40	60	50	50	100	0
Gentamycin	5	95	0	100	12.5	87.5	0	100	0	100	16.7	83.3	25	75
Imipenum	0	100	0	100	0	100	0	100	0	100	0	100	0	100
Ofloxacin	10	90	10	90	0	100	0	100	0	100	16.7	83.3	0	100
Piperacillin/ Tazobactam	0	100	0	100	0	100	0	100	0	100	0	100	0	100
Tobramycin	5	95	0	100	0	100	0	100	0	100	0	100	0	100

Table 2. Antimicrobial resistance patterns of gram-positive bacteria isolated in eye swab cultures of newborn babies

Gram-positive sensitivity testing results	Staphylococcus species (n=16)		Micrococcus species (n=1)		Corynebacterium species (n=2)	
	Resistant %	Sensitive %	Resistant %	Sensitive %	Resistant %	Sensitive %
Amikacin	0	100	0	100	0	100
Chloramphenicol	18.7	81.3	0	100	50	50
Clindamycin	18.7	81.3	0	100	50	50
Cloxacillin	50	50	100	0	0	100
Cotrimoxazole	31.3	68.7	0	100	50	50
Erythromycin	56.3	43.7	0	100	0	100
Fucidic acid	31.3	68.7	0	100	0	100
Gentamycin	31.3	68.7	0	100	0	100
Ofloxacin	18.7	81.3	0	100	0	100
Penicillin	87.5	12.5	0	100	0	100
Tetracycline	43.7	56.3	0	100	0	100
Vancomycin	0	100	0	100	0	100