Purpose: The study aimed to determine the infective spectrum and sensitivity testing in cases of Ophthalmia neonatorum (ON) at Kenyatta National Hospital and Pumwani Maternity Hospital in Nairobi, Kenya. Methods: 120 neonates with ON were studied. Conjunctival swabs were taken for culture and conjunctival scrapings were performed for Direct immunofluorescence to detect Chlamydia trachomatis. Results: In 110 (91.6 %) neonates with ON cultures were positive. Neisseria gonorrhoeae was isolated in 4 patients. All isolates were resistant to Penicillin. In 24 (20 %) cases Chlamydia trachomatis could be detected. Staph. aureus (35.8 %), Staphy.epidermidis (28.3 %) and E. coli (11.7%) were the most common cultured bacteria. Staphylococcus spp. were resistant to Tetracycline, Penicillin Kanamycin and sensitive to Ofloxacin, Amikacin, Neomycin and Gentamycin. E. coli was resistant to Penicillin, Cephalexin, Polymyxin B and sensitive to Ofloxacin and Aminoglycosides. In only 52.5 % prophylaxis treatment was instituted after birth. Conclusions: In our study Chlamydia trachomatis accounted for 20 % of all infections. The incidence of Neisseria gonorrhoeae was low. Tetracycline, Penicillin and Kanamycin the commonly used drugs in our set up in Nairobi. were found to be resistant to most of the isolated bacteria. Therefore an antiseptic prophylaxis like Povidone iodine should be used. All neonates should receive prophylaxis for ON and health workers should be sensitized on need for ocular prophylaxis.