Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 4 Issue 11 November 2022

Case Report

Corneal Infection in Rabbits Caused by *Pseudomonas Aeruginosa*: A Case Report

Kareem O Kafilat[1]*, Aina O Oluwagbemiga[1], Enwuru A Christian[2], Awoderu B Oluwatoyin[2] and Bamgbose J Damilare[1]

[1]*Biochemistry and Nutrition Department, Nigerian Institute of Medical Research, Nigeria*

[2]*Center for Infectious Disease Research Laboratory, Nigerian Institute of Medical Research, Nigeria*

***Corresponding Author:** Kareem O Kafilat, Biochemistry and Nutrition Department, Nigerian Institute of Medical Research, Nigeria.

Received: September 19, 2022

Published: October 04, 2022

Abstract

New Zealand White rabbits were presented with corneal opacity and watery discharge from the eyes. Repeat syndromic management using antimicrobial treatment with gentamicin did not lead to clinical improvement. Using standard protocols, a definitive diagnosis was made with microbiological culture and antibiogram at the Center for Infectious Disease Research laboratory. Biochemical analysis revealed *Pseudomonas aeruginosa* as the etiologic agent, *and it has been* reported as a common organism associated with bacterial keratitis. The antibiotic susceptibility tests showed sensitivity to piperacillin, ceftazidime, and imipenem, which are recommended choices for Pseudomonas. However, the animals were subjected to a second-line antibiotic (Ciprofloxacin) treatment for 7 days, and by the 5th day, the symptoms were relieved. A hematological examination showed neutropenia and lymphopenia. The findings of this report underscore the need for microbial isolation, identification, and susceptibility testing before the medical armamentarium.

Keywords: Corneal Infection; Rabbits; *Pseudomonas Aeruginosa*

Introduction

Pseudomonas aeruginosa is an opportunistic bacterial pathogen, commonly found in the environment (soil and water). Previous studies linked the organism to corneal infection, which was characterized by fulminating corneal destruction caused by *in vivo* production of extracellular corneal-damaging pseudomonal proteases [1]. *Pseudomonas aeruginosa* has a worrisome characteristic as it is resistant to many antibiotics, including aminoglycosides, quinolones, and beta-lactams [2]. The proteases of *P. aeruginosa* that have been implicated as being essential for ocular virulence include exotoxin A, phospholipase C, LasB (elastase), alkaline protease, and the LasA protease [3]. Pseudomonal corneal ulcers are described as rapidly progressive with extensive tissue destruction. Proinflammatory cytokines and chemokines, as well as an influx of polymorphonuclear leukocytes [4], are thought to be active in corneal inflammation. Unlike other types of bacterial keratitis, the infection caused by *P. aeruginosa* is especially virulent and sight-threatening [5].

The current case presents the disease progression, difficulties, and limitations in the treatment of pseudomonas corneal infections in rabbits. Also, we report the measures taken to alleviate the

inflammation and discharge from the affected eyes of the animals and stress the potential health risk of the causative agent.

Case Presentation

Adult New Zealand White (NZW) rabbits were purchased for research purposes and kept at the Nigerian Institute of Medical Research animal house. Prior to this case, all animals were healthy and showed no signs of morbidity. They were maintained under a normal dark and light cycle, with feed and fresh water given ad libitum. Three months post arrival, one of the NZW rabbits had mucous discharge from the right eye, which quickly spread to the left and led to the closure of both eyelids. He became weak due to inappetence. In two weeks, the infection spread to 20 other rabbits. The sick ones were separated from the healthy ones to control the spread of infection, and the cages were fumigated with formalin and ammonia in a ratio of 1:10. Each rabbit was handled with care, and the eyes were cleaned with cotton wool and distilled water to remove mucous that had become lodged on the eyelid, allowing the rabbit to see their surroundings, depending on the stage of infection they were in. Four different stages were identified: central corneal ulcer, advanced corneal lesion, liquefaction necrosis, and purulent exudate (Figure 1-4). During the central corneal stage, there was mild conjunctivitis and corneal opacity initially; this progressed rapidly to severe conjunctivitis with purulent exudates within 24hrs. The cornea had a protruding fluid-filled sac-like lesion. In the final stage, the corneal ulcers were seen grossly with extensive liquefaction necrosis. The corneas of rabbits at this stage were markedly edematous and coated with purulent exudate that appeared as a protruding milky-white gel.

Figure 1: Small central corneal ulcer in rabbit.

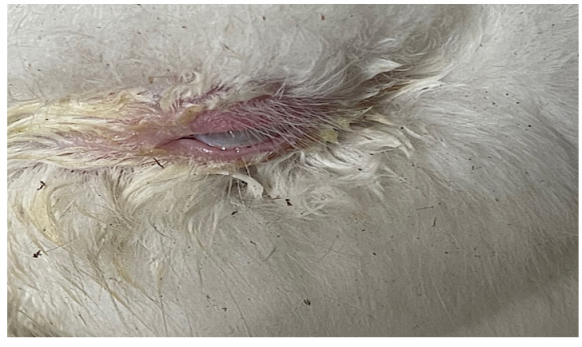

Figure 2: Advanced corneal lesion stage.

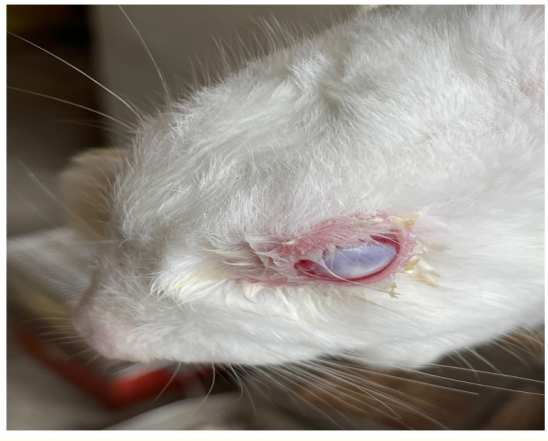

Figure 3: Liquefaction necrosis stage.

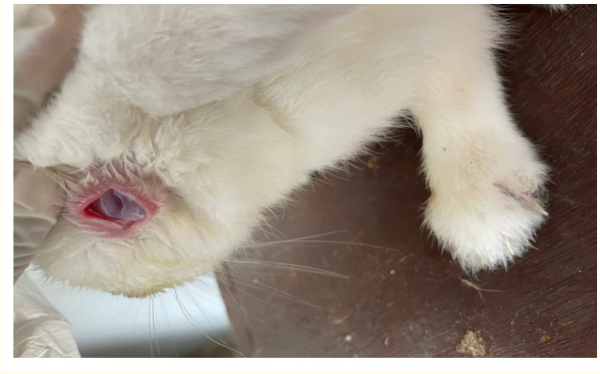

Figure 4: Extensive liquefaction necrosis and purulent exudate.

Investigation

During the peak infection period, rectal temperature was taken daily and averaged 40° C. Blood samples were collected into heparinized tubes for hematological analysis with a hematology autoanalyzer. The ocular discharge from four severely affected rabbits was sampled with a sterile swab directly from the cornea and submitted to the laboratory for investigation. The swabs were collected, labeled, and immersed in 2 vials of Brain Heart Infusion (BHI) and a vial of Liver Infusion Broth for each sample. Two of the inoculums were incubated aerobically for 24 hours, while the other BHI vial was incubated anaerobically. Loops of the incubated broths were inoculated onto MacConkey Agar, Blood Agar, and Chocolate Agar and incubated aerobically and anaerobically for 24 hours at 37°C. The anaerobic culture yielded no growth, while mild to moderate non-lactose fermenter bacterial growth was observed on the MacConkey agar of all the samples. Gram stain microscopy results yielded gram-negative bacilli, while BioMic V^3. (Becton Dickson, USA), a semi-automated bacteriological identification system, identified the isolates as *P. aeruginosa*.

Subsequently, all isolates were subjected to *in vitro* antimicrobial susceptibility testing against commonly used antibiotics (amikacin, piperacillin, imipenem, cefuroxime, cefepime, trimethoprim-sulfamethoxazole, gentamicin, cefixime, tetracycline, erythromycin, ofloxacin, cefotaxime, meropenem, ceftazidime/clavulanic acid and also ciprofloxacin) (Table 1). The bacteria were within the susceptibility ranges of the Clinical Laboratory Standard Institute (CLSI) [6,7] interpretative criteria for Imipenem and Ciprofloxacin. The hematology report (Table 2) showed neutropenia and lymphopenia in three of the samples tested.

Sample	**Sample A**	**Sample B**	**Sample C**	**Sample D**
Antibiotics/Range(mm)	**zone of inhibition (mm)/Interpretation**			
Amikacin (S: ≥ 19; I: 16-18)	(19) S	(20) S	(16) I	(20) S
Piperacillin (S: ≥19; I: 11-18)	(17) I	(23) S	(24) S	(22) S
Imipenem (S: ≥ 20; I: 17-19)	(20) S	(24) S	(23) S	(22) S
Cefuroxime (S: ≥23; I: 14-22)	(0) R	(0) R	(0) R	(8) R
Cefepime (S: ≥32; I: 27-31)	(25) R	(26) R	(23) R	(28) I
Ampicillin (S: ≥ 15; I *)	(0) R	(0) R	(0) R	(6) R
Trimethoprim-Sulfamethoxazole (S: ≥26; I: 23-25)	(0) R	(6) R	(7) R	(6) R
Gentamicin (S: 26; I: 23-25)	(17) R	(15) R	(11) R	(15) R
Cefixime (S: ≥20; I: *)	(0) R	(0) R	(0) R	(0) R
Tetracycline (S: 26; I: 23-25)	(7) R	(0) R	(0) R	(0) R
Ciprofloxacin (S: ≥ 21; I: 15-20)	(30) S	(30) S	(30) S	(31) S
Erythromycin (S: ≥ 23; I: 16-22)	(9) R	(0) R	(0) R	(0) R
Ofloxacin (S: ≥22; I: 16-21)	(17) I	(16) I	(15) R	(17) I
Cefotaxime (S: ≥23; I: 18-22)	(20) I	(0) R	(15) R	(12) R
Meropenem (S: 27; I: 20-26)	(20) I	(15) R	(22) I	(22) I
Ceftazidime/Clavulanic acid (S: 19; I: 17 -18)	(22) S	(21) S	(17) I	(20) S

Table 1: Antibiotic susceptibility testing.

S: Susceptible; I: Intermediate; R: Resistant

* = Not indicated.

Parameter	A 1	A 2	A3	A4	A5	REF[7]
WBC × 10^3/L	4.25	4.13	4.55	4.35	3.09	5.2-12.5 x 10^6m^3
RBC × 10^3/L	3.15	4.12	5.06	5.32	4.46	5.1-7.9 x 10^6m^3
HGB × g/L	71	86	99	99	90	10.0- 17.4 g/dL
HCT %	0.202	0.25	0.315	0.307	0.283	33%-50%
MCV FL	57.5	60.7	62.3	57.7	63.5	57.8- 66.5
MCH pg	20.2	20.9	19.6	18.6	20.2	17.1-23.5 pg
MCHC g/L	351	344	314	322	318	29%- 37%
PLT × 10^3/L	248	257	242	258	76	250-650 x 10^6 m^3
NEUT × 10^3/L	2.74	2.60	1.61	1.30	1.77	2.0 -7.5 10^3/L
LYMP × 10^3/L	1.12	1.00	2.56	2.71	1.15	*
Lymph %	26.4	24.2	56.3	62.3	37.2	30%-85%
Mono × 10^3/L	0.13	0.31	0.16	0.10	0.10	*
Eos × 10^3/L	0.00	0.00	0.00	0.00	0.01	*
Bas × 10^3/L	0.26	0.22	0.22	0.24	0.06	*

Table 2: Hematology result of blood samples from the rabbits.

WBC: White Blood Cells; RBC: Red Blood Cells; HGB: Hemoglobin; HCT: Hematocrit; MCV: mMean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; PLT: Platelet; LYMPH: Lymphocytes; MONO: Monocytes; EOS: Eosinophils; BAS: Basophils; NEUT: Neutrophils

*=Not available.

Treatment

They were treated with 2.5mg/kg BW gentamicin IM for 5 days and with gentamicin eye drops as conjunctivitis was suspected. Following the diagnosis of *P. aeruginosa* infection with culture and after the susceptibility testing results were available in August 2021, the rabbit treatment was modified to ophthalmic ciprofloxacin 3 mg/ml, administered every minute for 5 minutes and then every 30 minutes for the first day, and then thrice a day for 7 days. Those with advanced stages of the disease condition had, in addition to the ophthalmic application, ciprofloxacin IV 20 mg/kg administered to them. After one week of treatment, none of the rabbits died; the eye symptoms had regressed to stage one, and they eventually had almost clear pupils.

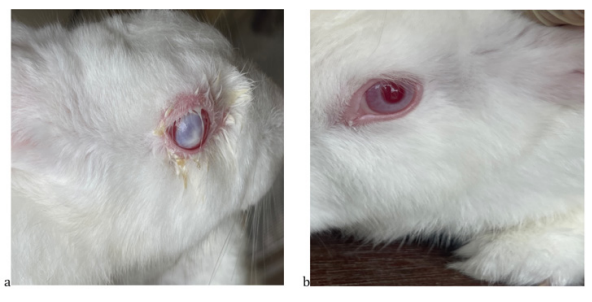

Figure 5: a and b. during infection phase and post-infection in a rabbit.

Outcome and follow-up

At the time of writing this report, reinfection had occurred in 2 rabbits, and they were subsequently treated appropriately. The infection phase and post-infection in a rabbit of one infected rabbit are shown in figures 5a and b.

Discussion

Bacterial infection of the cornea threatens vision, creating ocular emergencies that require immediate therapy with an effective antimicrobial agent [8]. *P. aeruginosa* is an opportunistic pathogen in humans and has been reported as one of the most frequently isolated bacterial species in contact lenses [9]. It is usually associated with keratitis and serious corneal infections in animals. In line with our observations, the lesions are said to be extensive and progress rapidly. The increasing incidence of keratitis related to multidrug-resistant *P. aeruginosa* has been well documented [10]. This report agrees with several previous reports on Pseudomonas resistance to several antibiotics and shows near-consistent sensitivity to ciprofloxacin. Antibacterial resistance resulted in the mortality of seven rabbits after failed treatment with gentamicin. However, gentamicin resistance has been reported severally. This case agrees with the report of Garg., *et al*. [8], who reported successful treatment with ophthalmic antimicrobial therapy. In this case, specifically, the rabbits were treated with ophthalmic and IV ciprofloxacin ragmen, and the outcome was excellent. Saee., *et al.* [11] in their report have it that microbial keratitis caused by gram-negative bacteria, particularly the pseudomonas species, tends to be severe and rapidly progressive, and our observations in the course of managing this case agree with these assertions. Their report further ascribed the disease's rapid progressive tendency to the organism's complex structure and multiple virulence factors. Pseudomonas species have been reported to pose a challenge to the host's immune system and can cause sepsis with a fatality rate of 37% to 77% [12]. Early antibiotic administration is critical for optimal survival rates in neutropenic animals [12]. The latter most probably contributed to our favorable outcome in this early antibiotic intervention, especially when early therapy is said to be essential in pseudomonas infection management because the administration of systemic or subconjunctival antibiotics is ineffective if the infection is already established [13]. Melillo (2007) [14] reported that fever, increased plasma cortisol concentrations, neutrophilia, and lymphopenia were observed in rabbits injected with bacteria or yeast experimentally. This corroborates our report of fever, neutropenia, and lymphopenia, although we did not measure plasma cortisol in this case report. Since *P. aeruginosa* has been severally reported to occupy a wide range of environmental niches, the bacterial contamination was suspected to be the source of water supply for the animals, and the source has since been treated.

Conclusion

In conclusion, pseudomonas septicemia is rapidly fatal if not treated promptly with appropriate antibiotics. Imipenem, Ceftazidime/Clavulanic acid, and Ciprofloxacin showed good sensitivity in this case. Many pathogens are now resistant to conventional antibiotics, so a routine antibiogram profile should be performed before administering antibiotics to prevent further resistance from spreading. Also, it is apt to note that not every rapidly progressing disease in an animal is of viral origin. The culture of corneal samples and antimicrobial drug sensitivity testing are important tools in dealing with this infection because a thorough investigation is required to make a diagnosis and treatment plan.

Conflict of Interest

The authors declare they have no conflict of interest.

Funding

The authors declared they received no funding for this report.

Bibliography

1. Gray LD and Kreger AS. "Rabbit corneal damage produced by Pseudomonas aeruginosa infection". *Infection and Immunity* 12.2 (1975): 419-432.

2. Hancock RE and Speert DP. "Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and impact on treatment". *Drug Resistance Updates* 3.4 (2000): 247-255.

3. Preston MJ., *et al*. "Contribution of proteases and LasR to the virulence of Pseudomonas aeruginosa during corneal infections". *Infection and Immunity* 65.8 (1997): 3086-3090.

4. Alionte LG., *et al*. "Pseudomonas aeruginosa LasA protease and corneal infections". *Current Eye Research* 22.4 (2001): 266-271.

5. Hobden JA. "Pseudomonas aeruginosa proteases and corneal virulence". *DNA and Cell Biology* 21.5-6 (2002): 391-396.

6. Clinical Laboratory Standard Institute (CLSI) 30th edition. Performance Standards of Antimicrobial Susceptibility Testing (2020).

7. Benson KG and Paul-Murphy J. "Clinical pathology of the domestic rabbit: acquisition and interpretation of samples". *Vet-*

erinary Clinics of North America: Exotic Animal Practice 2.3 (1999): 539-551.

8. Garg P., *et al*. "Ciprofloxacin-resistant Pseudomonas keratitis". *Ophthalmology* 106.7 (1999): 1319-1323.

9. Lomholt JA., *et al*. "Epidemic population structure of Pseudomonas aeruginosa: evidence for a clone that is pathogenic to the eye and that has a distinct combination of virulence factors". *Infection and Immunity* 69.10 (2001): 6284-6295.

10. Vazirani J., *et al*. "Multidrug-resistant Pseudomonas aeruginosa keratitis: risk factors, clinical characteristics, and outcomes". *Ophthalmology* 122.10 (2015): 2110-2114.

11. Saeed A., *et al*. "Risk factors, microbiological findings, and clinical outcomes in cases of microbial keratitis admitted to a tertiary referral center in Ireland". *Cornea* 28.3 (2009): 285-292.

12. Bodey GP., *et al*. "Infections caused by Pseudomonas aeruginosa". *Reviews of Infectious Diseases* 5.2 (1983): 279-313.

13. Peyman GA., *et al*. "Postoperative endophthalmitis: a comparison of methods for treatment and prophylaxis with gentamicin". *Ophthalmology Surgery* 6.1 (1975): 45-55.

14. Melillo A. "Rabbit clinical pathology". *Journal of Exotic Pet Medicine* 16.3 (2007): 135-145.