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Experimental Investigation on Rabbits Following Peritoneal Injection of Bacteria Isolated from Human Keratitis

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Authors' contributions

All authors contributed and cooperated in designing and running the experiment as well as writing the article. Author SMA studied the histopathology. Author AAK studied the biochemistry. Authors YHA and MIA followed up the clinical signs of infected rabbits during the experiment. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: This work was aimed at studying the pathogenicity of bacteria causing infectious keratitis through experimental infection using different groups of rabbits that were inoculated with clinical isolates and assessed through biochemical and histopathological investigations.
Study Design: This study was carried out on Rabbits.
Place and Duration of the Study: this experiment was carried out at Med. Labs. Dept, Qassim Univ., in April 2013.
Methodology: The isolated bacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus* from clinical corneal scraping swabs of patients suffering from infectious keratitis were experimentally inoculated through intraperitoneal injection in different groups of

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rabbits (2.0-2.5kg) and were subjected to serum biochemical and histopathological examinations.
Results: The experimental rabbits showed alterations in both liver and kidney function parameters that varied with the type of bacteria injected. The level of urea was non-significantly increased to a higher extent in rabbits within 3 and 7 days of infection. There was no marked change in the levels of uric acid and creatinine in all groups of rabbits. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) showed variable increased values but seemed also within limits of reference in all the groups of rabbits injected with either *S. aureus* or *P. aeruginosa.*
Histopathologically, the internal organs (liver and kidneys) of the experimental rabbits showed inflammatory reactions with degenerative changes and/or necrosis while the cornea revealed oedema and leukocytic infiltration. The microscopic findings were varied in severity according to the type of the bacteria.
Conclusion: *Pseudomonas aeruginosa* and *Staphylococcus aureus* experimentally induced infections revealed histopathologic lesions and disturbances in the functions of liver and kidneys of experimental rabbits together with proliferation of corneal epithelium and polymorphonuclear leukocytes infiltration in the corneal stroma. Therefore, strict measures are recommended to control and treat infectious keratitis to avoid visual complications and systemic disturbances among infected patients.

Keywords: Rabbits; Infectious keratitis; Pseudomonas aeruginosa; Staphylococcus aureus; liver function tests; kidney function tests; histopathology.

1. INTRODUCTION

Ocular infection may lead to a significant reduction in visual functions. Corneal abrasions can come from mechanical injuries such as contact lens, foreign body, finger nails, twigs or laser refractive surgery. Bacterial keratitis is a very common and significant cause of ocular morbidity that can result in severe visual loss. So, early determination of the causative infective organism is essential for the effective treatment [1-2].

Potential biological infectious microbes of keratitis include fungi, viruses, and protozoa; however, most corneal infections are associated with bacteria [3]. Several ocular disorders are associated with different Gram positive and Gram negative bacteria, including *Staphylococcus aureus*, *Streptococcus* spp., *Bacillus subtilis*, *Rhodococcus* spp., *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Haemophilus aegyptius* and *Klebsiella spp.* [4-6].

In spite of many advances in diagnosis, management and availability of potent antibiotics, microbial keratitis spreads very commonly as these bacteria can proliferate rapidly by their devastating enzymes like coagulases, collagenases, lipases and exotoxins. It is not common to bacteria and its toxins to penetrate the ocular barrier to various organs except in severe cases, however severe keratitis can potentially lead to blindness or even loss of entire eye and in many cases surgical intervention are needed [7-8].

Recent studies of bacterial keratitis from different parts of the world have found coagulase negative Staphylococcus (CNS) to be the most common pathogen [9-11]. However, others have found *P. aeruginosa* to be the most common pathogen overall [12-13]. There is extensive variability with regard to the distribution and frequency of the different pathogens, as well as their antibiotic resistance profiles among series from different locations.

Several other rare types of keratitis include non-tuberculosis mycobacterial keratitis. This type of keratitis commonly occurs after trauma or refractive surgery and looks like fungal, herpetic or amoebic keratitis [14]. In addition to this there can be higher chances of bacterial keratitis among the patients suffering from diabetes mellitus [15].

In our previous related study, the collected corneal scraping swabs of patients suffering from infectious keratitis revealed the isolation of bacteria from 63 swabs (54.8%) where *P. aeruginosa* (n=29, 25.2%), *S. aureus* (n=18, 15.7%), and unclassified bacteria (n=16, 13.9%) were isolated [16].

Different types of bacterial and other microbial infections in humans alter the homeostasis by producing different types of toxins that request to detect the status of the primary function of the liver and kidneys which is to expel different types of toxins that are produced as a result from the body's metabolism [17,18]. So, malfunction of the liver and kidney can translate to serious conditions that may be life threatening.

This present research aimed to study the pathogenicity of *P. aeruginosa* and *S. aureus,* isolated from the cornea through experimental infection by investigating the liver and kidney functions via biochemical and histopathological examinations.

2. MATERIALS AND METHODS

2.1 Bacteria

Bacteria **(***Pseudomonas aeruginosa* and *Staphylococcus aureus)* were provided as reference isolates by Bacteriology Lab., Medical Laboratory Dept., College of Applied Medical Sciences, Qassim University. These two bacteria were isolated from corneal scraping swabs of patients suffering from infectious keratitis in our previous related study and subjected to standard technique for isolation and identification and confirmation of the isolates API system (Bio Merieux) were carried out. The bacteria were stored in a mixture of trypticase soy broth (TSB) and trypticase soy agar (TSA) supplemented with glycerol (15%) at - 86ºC to be used in this experimental infection.

2.2 Animals

This experimental study was conducted on 27 male albino rabbits weighing between 2.0-2.5kg, obtained from the veterinary farm of Qassim University. The rabbits were properly examined and proved free from any disease. The rabbits were allowed to acclimatize for 7 days in the animal house before starting the experiment. The experimental animals were housed in air conditioned rooms at18-20ºC and 60-65% relative humidity and kept on 12h light/dark cycle. They were housed in standard aluminum cages and fed with standard rabbit diet and normal tap water. All authors hereby declare that “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) were followed, which was also in compliance with the experimental laboratory animals welfare act, College of Applied Medical Sciences, Qassim University. Our experiment protocol was implemented after the clearance from Animal Ethical Committee, Qassim University.

2.3 Experimental Design

This experimental study was done at Med. Labs Dept, Qassim Univ., during April of 2013. Rabbits were chosen as a model for the experiment because it is easier to follow the diseases in cornea like infectious keratitis, corneal ulcer and corneal slit lamp photography. The Rabbits were divided randomly into three equal groups, each containing 9 rabbits. The experimental infection was done through the intraperitoneal (IP) inoculation for two reasons; first it is not common for the bacteria to penetrate the ocular barrier to various organs except in rare severe cases, second we aimed to study the possibility of eye involvement upon systemic infection (IP inoculation) and also to check the systemic effect of these bacteria.

Group I: Rabbits as negative control group were inoculated intraperitoneally (IP) with sterile buffer saline.
Group II: Rabbits were IP inoculated with isolated *Pseudomonas aeruginosa*.
Group III: Rabbits were IP inoculated with isolated *Staphylococcus aureus*.

Bacterial inoculums were prepared by cultivating each bacterial species onto nutrient agar for 24 h at 37ºC, and then 5-7 colonies were transferred to a tube containing 5 ml sterile normal saline solution. The tubes were vortexed to make a bacterial suspension with turbidity equal to 0.5 McFarlands standard solution (equivalent to 5×10^8 colony-forming units (CFU/mL). The suspension was then adjusted to a final concentration of 10^5 CFU/mL, as verified by a quantitative bacterial count on Mueller-Hinton agar plates. A bacterial suspension of 2ml from each sample was carefully injected intraperitoneally to each experimental animal using sterilized syringes.

2.4 Blood and Tissue Collection

Rabbits were observed by animal handling experts for any clinical signs of illness for two weeks. Euthanasia of rabbits was carried out by intravenous injection of 0.3ml per Kg of body weight of T-61 Euthanasia Solution® (Intervet) (Embutramide 200mg; Mebezonium Iodide 50mg; Tetracaine Hydrochloride 5mg/ml). After euthanasia, the animals were sacrificed on 3rd, 7th and 14th day of post-infection, to collect fresh blood samples that were kept 30 min. at room temperature to clot and the serum was isolated through centrifugation at 3000 rpm for biochemical examination and other tissue specimens (liver and kidney) were collected for histopathological investigation.

2.5 Biochemical Analysis

Blood samples (serum) were used for liver and kidney function analysis. Diagnostic kits for serum ALT, AST, urea, uric acid and creatinine were purchased from Human Diagnostic Kits (Human GmbH, Wiesbaden Germany) and were used according to manufacturer's instructions.

2.6 Histopathological Techniques

Tissue sections from the eyes and internal organs (liver and kidneys) of experimental rabbits were taken and immediately fixed in 10% neutral buffered formalin, then dehydrated in increasing concentrations of ethyl alcohol, cleared in xylene, blocked in paraffin and sectioned as 5µm using rotary microtome. The obtained tissue slides were stained with hematoxylin and eosin (H and E) [19].

2.7 Statistical Analyses

A probability at the level of 0.05 was considered significant. Means and standard deviation were also estimated. All statistical analyses were run on the computer, using the SAS program (SAS, 2003).

3. RESULTS

3.1 Clinical Findings in Experimental Models

The intraperitoneal injection of rabbits with either *Psudomonas aeruginosa* or *Staphylococcus aureus* caused lethargy, pain and diarrhea. Clinically, the rabbits injected intraperitoneally with *Pseudomonas aeruginosa* became very sick within 3-4 days and showed mild corneal haze (Fig. 1) while those infected with *Staphylococcus aureus,* were apparently normal. All of the rabbits survived the bacterial inoculation till the end of the experimental period.

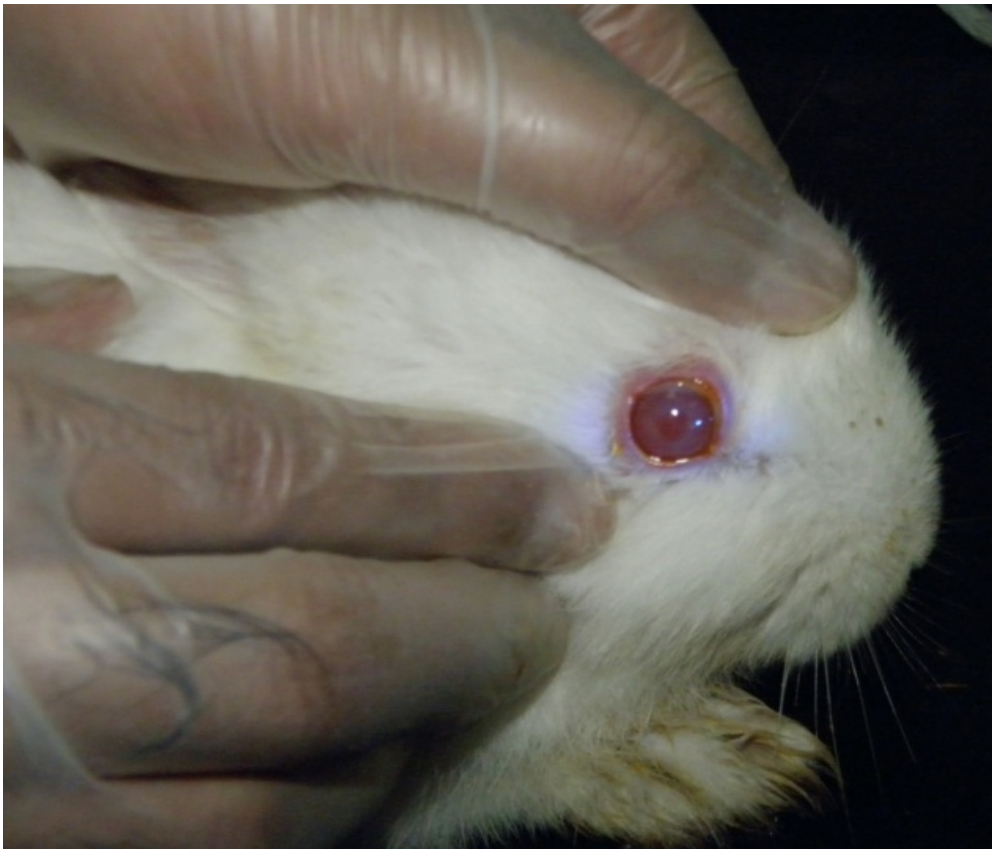

Fig. 1. Rabbit, experimentally I/P inoculated with *Psudomonas aeruginosa,* showing mild corneal haze

3.2 Biochemical Profile

The level of urea increased in blood of rabbits after 3 and 7 days of intraperitoneal injection of *Staphylococcus aureus* and *Pseudomonas aeruginosa*as as shown in (Table 1). The level of urea was increased to a higher extent after two weeks of intraperitoneal injection with *Staphylococcus* as compared to *Pseudomonas* injection. There was no marked change in the level of uric acid and creatinine in all groups that intraperitoneally were injected either with *S. aureus* or *P. aeruginosa.* Although ALT and AST showed unremarkable increased values, they were also within limits of reference in all the groups of rabbits injected with either *S. aureus* or *P. aeruginosa.*

3.3 Histopathology

The normal histological structure of rabbit's cornea is shown in (Fig. 2).

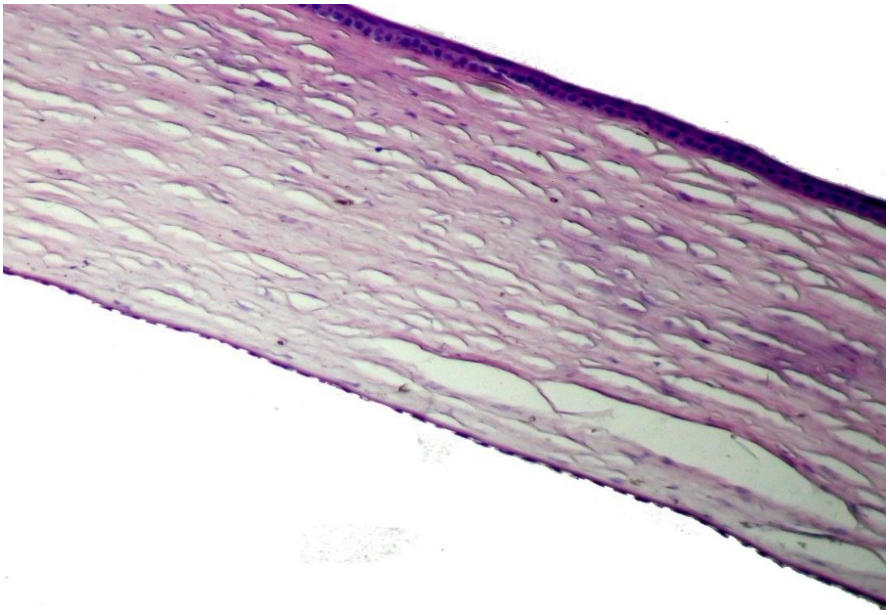

Fig. 2. Cornea, of control group, showing the 5 layers of cornea epithelium, basement membrane, stroma, Descement's membrane and endothelium. H & E stain, x 40

3.3.1 Rabbits IP inoculated with *Pseudomonas aeruginosa*

At the 7th day of IP injection, oedema in the corneal subepithelial tissue and congestion, vacuolar degeneration as well as polymorphonuclear leukocytes in the liver and kidneys were evident (Fig. 3). At the 14th day of IP injection, the corneal stroma was oedematous and infiltrated with polymorphonuclear leukocytes (Fig. 4). The liver showed mild form of degeneration (cloudy swelling) in the hepatocytes while the kidneys exhibited tubular nephrosis mainly vacuolar degeneration in the renal tubules.

Table 1. Different biochemical parameters in Rabbits intraperitoneally infected with bacteria for the evaluation of liver and kidney function parameters

Day of inoculation	IP sample	Urea (mg/dL)	*Uric acid (mg/dL)*	Creatinine (mg/dL)	ALT (U/L)	AST (U/L)
	Control	29.53±3.54	*0.64±0.08*	0.87±0.12	27.92±1.45	14.59±1.21
3	*Pseudomonas aeruginosa*	43.81±3.23	*0.24±0.01*	0.57±0.11	26.33±1.26	16.34±1.87
	Staphylococcus aureus	42.35±2.47	*1.03±0.06*	0.84±0.20	32.05±2.21	13.32±1.22
7	Control	15.09±1.98	*0.53±0.09*	1.08±0.02	24.75±3.10	9.20±1.98
	Pseudomonas aeruginosa	17.46±1.68	*1.84±0.04*	1.92±0.07	27.87±2.14	12.22±1.20
	Staphylococcus aureus	18.33±2.13	*0.76±o.08*	1.01±0.08	27.92±1.18	14.87±2.13
14	Control	31.96±3.45	*0.42±0.07*	0.77±0.04	24.87±1.76	15.87±2.76
	Pseudomonas aeruginosa	26.28±2.87	*0.24±0.03*	0.94±0.12	29.19±2.25	13.73±1.54
	Staphylococcus aureus	50.46±0.86	*0.54±0.08*	0.67±0.015	33.80±2.12	18.88±0.34

Values are mean ± SD (range), a probability at the level of 0.05 or less was considered significant. Number of rabbits used were 27. P -value is significant at P<0.05, N=27, n=9

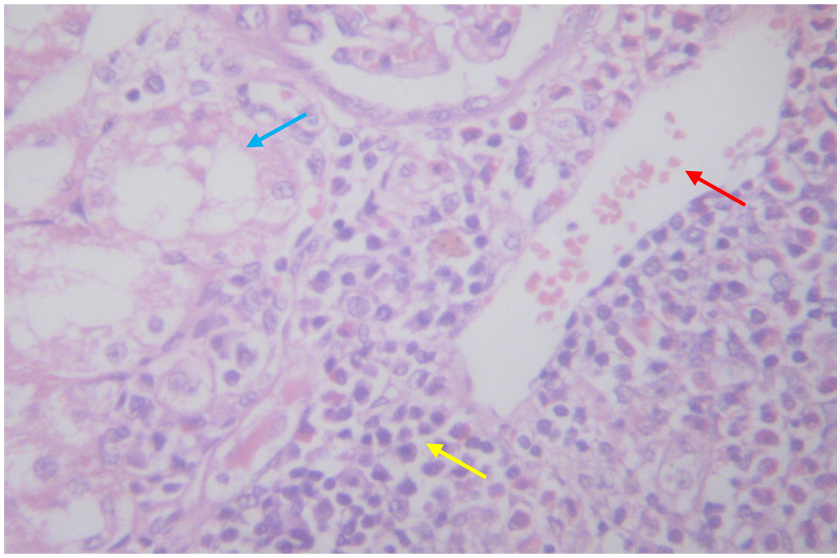

Fig. 3. Kidney, of rabbit at 7th day of IP inoculation with *Pseudomonas aeruginosa*, showing congestion of intertubular blood vessels (Red arrow), vacuolar degeneration (blue arrow) as well as polymorphonuclear leukocytes (yellow arrow) H & E stain, X 400

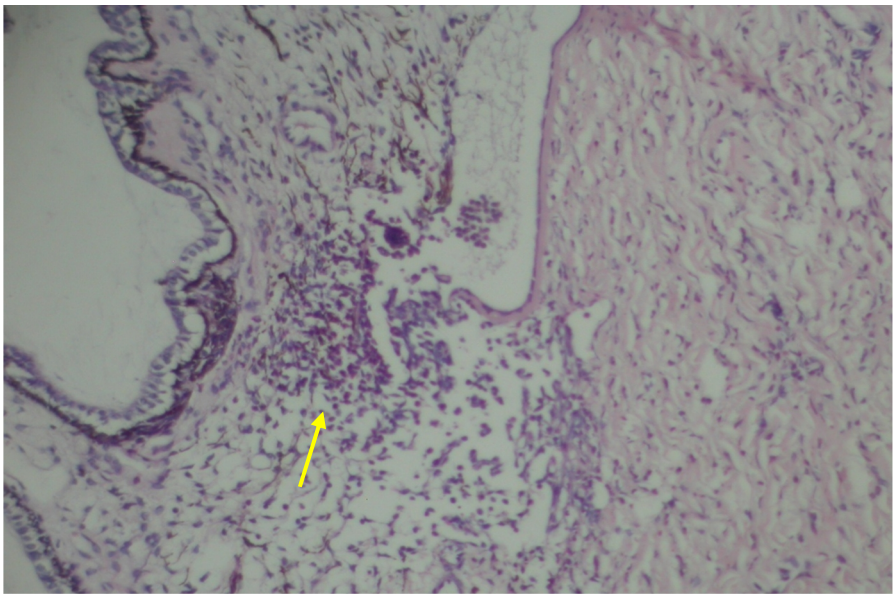

Fig. 4. Eye, of rabbit *a*t 7th day of IP inoculation with *Pseudomonas aeruginosa*, showing corneal stromal oedema and polymorphonuclear leukocyte infiltration (yellow arrow). H & E stain, X 250

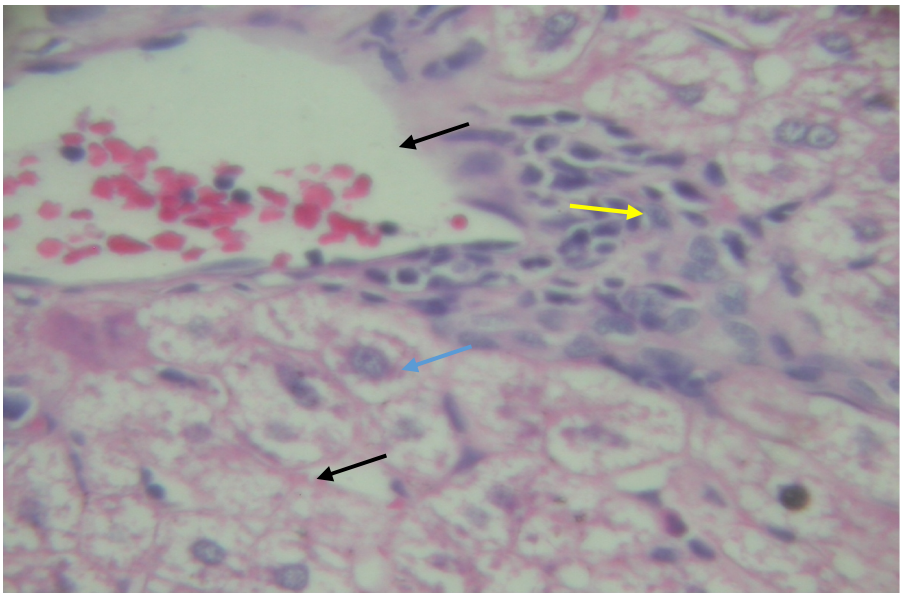

Fig. 5. Liver, of rabbit *a*t 7th day of IP inoculation with *Staphylococcus aureus*, showing congestion of central vein (black arrow), vacuolar degeneration (blue arrow), coagulative necrosis (black arrow) and polymorphonuclear leukocytes (yellow arrow). H & E stain, X 400

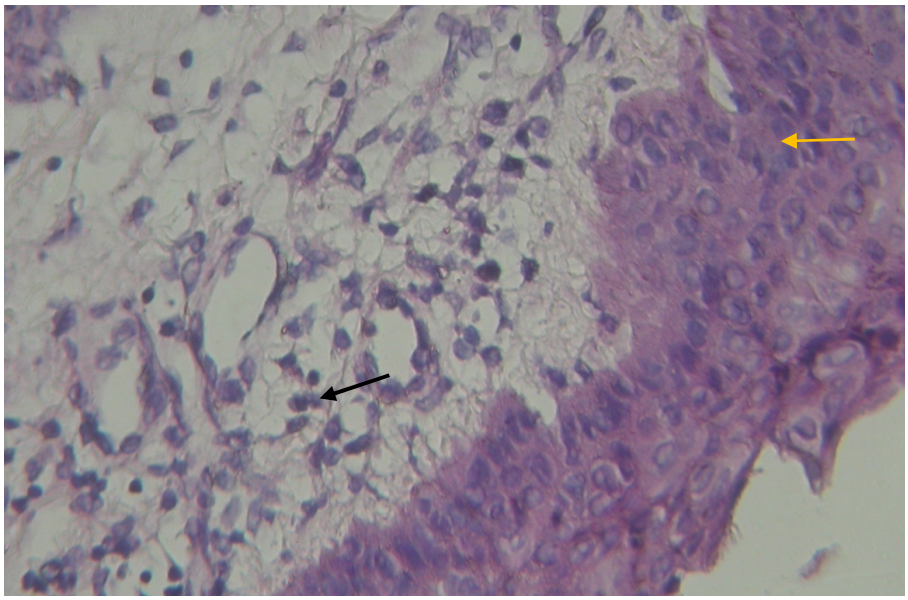

Fig. 6. Eye, of rabbit *a*t 7th day of IP inoculation with *Staphylococcus aureus*, showing proliferation of corneal epithelium (yellow arrow) and polymorphonuclear leukocytes infiltration in the corneal stroma (black arrow). H & E stain, X 250

3.3.2 Rabbits IP inoculated with *Staphylococcus aureus*

At 7th day of IP injection, focal desquamation in the corneal epithelium was evident with polymorphonuclear leukocytes in the stroma, this in addition to congestion, vacuolar degeneration, coagulative necrosis and polymorphonuclear leukocytes in the liver and kidneys (Fig. 5 above). At 14th day of IP injection, proliferation of corneal epithelium and polymorphonuclear leukocytes infiltration in the corneal stroma was observed (Fig. 6 above). Also, vacuolar degeneration and focal necrosis were seen in the hepatocytes while the kidney revealed cloudy swelling in the renal epithelium.

4. DISCUSSION

Staphylococcus aureus plays a major role among the hospital-acquired and other healthcare-associated bacteremia cases. The clinical course of *S. aureus* bacteremia is variable and difficult to predict [20]. As shown in Table 1, the level of urea increased to a higher extent within 3 days of the intraperitoneal injection of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The level of urea was non-significantly increased to a higher extent after two weeks of intraperitoneal injection with *Staphylococcus* as compared to *Pseudomonas* injection. It has been reported by others also that seventy two hours after inoculation of streptococcal pyrogenic exotoxin B wild and mutant types, the BUN as well as AST and ALT levels start rising [21]. The increase in urea level, ALT and AST could be due to the bacterial activity and the recorded histopathological lesions in both kidneys and liver. This has also been confirmed previously [21].

In the current study, IP inoculation of rabbits with *Pseudomonas aeruginosa* or *Staphylococcus aureus* revealed edema or desquamation of corneal epithelium with polymorphonuclear leukocytes in the corneal stroma at 7th day PI while at 14th day PI, infiltration of polymorphonuclear leukocytes in the corneal stroma and proliferation of corneal epithelium were evident. Some authors have also shown that *Pseudomonas aeruginosa* infections cause acute inflammation and liquefaction necrosis of the cornea [22].

O'Callaghan et al. reported, *Staphylococcus aureus* corneal infection results in extensive inflammation and tissue damage [23]. On the other hand, the liver and kidneys of rabbits intraperitoneally inoculated with *Pseudomonas aeruginosa* or *Staphylococcus aureus* showed inflammatory reactions and degenerative as well as necrotic changes at 7 days while at 14 days of experiment, degenerative changes and focal necrosis were evident. A previous study has also reported that *P. aeruginosa* infection causing necrosis of hepatic parenchyma [24]. Another study showed that, *Pseudomonas aeruginosa* infection, in acute cases, cause a systemic infection due to Gram-negative bacteria septicemia, while in the subacute to chronic stages of the disease, multifocal necrosis with abscessation may be present in kidneys [25].

5. CONCLUSION

Although rabbits behaved physically normal during two weeks of experimental infection, a mild systemic disturbances were recorded through biochemical and histopathological examination. However, there were apparent signs of sickness apparent during the first three days of post-IP injection with both bacteria. *Pseudomonas aeruginosa* and *Staphylococcus aureus* experimentally induced infections revealed histopathologic lesions and disturbances in the functions of liver and kidneys of experimental rabbits together with proliferation of

corneal epithelium and polymorphonuclear leukocytes infiltration in the corneal stroma. Therefore, strict measures are recommended to control and treat infectious keratitis to avoid visual complications and systemic disturbances among infected patients.

CONSENT

Not applicable.

ETHICAL APPROVAL

The authors have obtained all necessary ethical approval from suitable Institutional or State or National or International Committee. This confirms either that this study is not against the public interest, or that the release of information is allowed by legislation.

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lichtinger A, Yeung SN, Kim P, Amiran MD, Iovieno A, Elbaz U, et al. Shifting trends in bacterial keratitis in Toronto: An 11-year review. Ophthalmol. 2012;119:1785-90.
2. Pham-Vang S, Hardten DR. Recurrent corneal erosions causing bacterial keratitis. Optometry 2008;79:505-11.
3. Weissman BA, Mondino BJ. Risk factors for contact lens associated microbial keratitis. Cont Lens Anterior Eye. 2002;25:3-9.
4. Liao HR, Lee HW, Leu HS, Lin BJ, Juang CJ. Endogenous *Klebsiella pneumonia* endophthalmitis in diabetic patients. Can J Ophthalmol. 1992;27:143-7.
5. Aristoteli LP, Bojarski B, Willcox MD. Isolation of conjunctival mucin and differential interaction with *Pseudomonas aeruginosa* strains of varied pathogenic potential. Exp Eye Res. 2003;77:699–710.
6. Sensoy D, Cevher E, Sarıcı A, Yılmaz M, Ozdamar A, Bergisadi N. Bioadhesive sulfacetamide sodium microspheres: Evaluation of their effectiveness in the treatment of bacterial keratitis caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa* in a rabbit model. Eur J Pharmaceut Biopharmaceut. 2009;72:487–95.
7. O'Brien TP. Management of bacterial keratitis: beyond exorcism towards consideration of organism and host factors. Eye. 2003;17:957-74.
8. Kaye S, Tuft S, Neal T, Tole D, Leeming J, Figueiredo F, et al. Bacterial susceptibility to topical antimicrobials and clinical outcome in bacterial keratitis. Invest Ophthalmol Vis Sci. 2010;51:362-8.
9. Orlans HO, Hornby SJ, Bowler IC. *In vitro* antibiotic susceptibility patterns of bacterial keratitis isolates in Oxford, UK: A 10-year review. Eye (Lond) 2011;25:489–93.

10. Pandita A, Murphy C. Microbial keratitis in Waikato, New Zealand. Clin Exp Ophthalmol. 2011;39:393-7.
11. Gopinathan U, Sharma S, Garg P, Rao GN. Review of epidemiological features, microbiological diagnosis and treatment outcome of microbial keratitis: experience of over a decade. Indian J Ophthalmol. 2009;57:273-9.
12. Green M, Apel A, Stapleton F. Risk factors and causative organisms in microbial keratitis. Cornea 2008;27:22-7.
13. Shalchi Z, Gurbaxani A, Baker M, Nash J. Antibiotic resistance in microbial keratitis: ten-year experience of corneal scrapes in the United Kingdom. Ophthalmol. 2011;118:2161-5.
14. Chu HS, Hu FR. Non-tuberculous mycobacterial keratitis. Clin Microb Infec. 2013;19:221-6.
15. Barry A. Weissman, Bartly J. Mondino. Risk factors for contact lens associated microbial keratitis. Cont Lens Ant Eye. 2002;25:3–9.
16. Aldebasi YH, Aly SM, Ahmad MI, Khan AA. Incidence and risk factors of bacteria causing infectious keratitis. Saudi Med J. 2013;34:179-83.
17. Dhainaut JF, Marin N, Mignon A, Vinsonneau C. Hepatic response to sepsis: interaction between coagulation and inflammatory processes. Crit Care Med. 2001;29:42-7.
18. Rowland A, Miners JO, Mackenzie PI. The UDP-glucuronosyltransferases: Their role in drug metabolism and detoxification. The Int J Biochem Cell Biol. 2013;45:1121-32.
19. Bancroft JD, Stevens A. The haematoxylin and eosin. Theory and practice of histological techniques. 4th ed, Ch 6, Churchill Livingstone, London, New York & Tokyo. 1996;99–112.
20. Kern WV. Management of Staphylococcus aureus bacteremia and endocarditis: progresses and challenges. Cur Opinion Infec Dis. 2010;23:346–58.
21. Kuo C-F, Luo Y-H, Lin H-Y, Huang K-J, Wu J-J, Lei H-Y, et al. Histopathologic changes in kidney and liver correlate with streptococcal pyrogenic exotoxin B production in the mouse model of group A streptococcal infection. Microb Pathogen. 2004;36:273-85.
22. Gray LD, Kreger AS. Rabbit corneal damage produced by *Pseudomonas aeruginosa* infection. Infec Immun.1975;12:419-32.
23. O'Callaghan RJ, Callegan MC, Moreau JM, Green LC, Foster TJ, Hartford OM, et al. Specific roles of alpha-toxin and beta-toxin during *Staphylococcus aureus* corneal infection. Infect Immun. 1997;65:1571-8.
24. Tzanakakis GN, Veronikis DK, Anastasiou ED, McCully KS, Dimitracopoulos G. Histopathological lesions produced by *P. aeruginosa* lipopolysaccharide in rats. J Exp Pathol. 1989;4:199-211.
25. Percy DH, Barthold SW. Pathology of Laboratory Rodents and Rabbits. 1993;1(37-8)2:85-86.

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