Journal of Clinical & Experimental Ophthalmology

Todokoro, J Clin Exp Ophthalmol 2016, 7:3
DOI: 10.4172/2155-9570.1000557

Case Report Open Access

Post-Operative Endophthalmitis Caused by the Nutritionally Variant Streptococcus *Granulicatella adiacens*

Daisuke Todokoro[1*], Kiyofumi Mochizuki[2], Kiyofumi Ohkusu[3], Ryuichi Hosoya[4], Nobumichi Takahashi[2], and Shoji Kishi[1,5]

[1]*Department of Ophthalmology, Gunma University School of Medicine, Japan*

[2]*Department of Ophthalmology, Gifu University School of Medicine, Japan*

[3]*Department of Microbiology, Tokyo Medical University, Japan*

[4]*Department of Clinical Laboratory, Gunma University School of Medicine, Japan*

[5]*Maebashi Central Eye Clinic, Japan*

[*]**Corresponding author:** Daisuke Todokoro, Department of Ophthalmology, Gunma University School of Medicine, 3-39-15 Showa-machi, Maebashi, Gunma, Japan, Tel: +81-27-220-8338; Fax: +81-27-233-3841; E-mail: dtodokor@gunma-u.ac.jp

Received date: Apr 27, 2016; **Accepted date:** May 25, 2016; **Published date:** May 30, 2016

Abstract

Objective: Bacterial endophthalmitis is among the most serious ocular infections. Nutritionally variant streptococci (NVS) are bacteria that are difficult to culture with standard media because of fastidious growth requirements. The cases of post-operative endophthalmitis caused by *Granulicatella adiacens*, one of the NVS were reported.

Method: Case report and review of literature

Results: In Case 1, a 77-year-old female, who had undergone repeated ocular surgeries including trabeculectomy in the left eye, had developed late-onset bleb-related endophthalmitis without bleb leakage. The best corrected visual acuity (BCVA) showed light perception. A vitrectomy was performed, following this procedure and topical, intravitreal and systemic antibiotics, the BCVA recovered to 18/20.

In Case 2, an 85-year-old male had developed acute postoperative endophthalmitis after cataract surgery in his left eye. The BCVA was light perception in the left eye. A vitrectomy and the removal of intraocular lens were performed. Inflammation was improved after the vitrectomy and administration of topical, intravitreal and systemic antibiotics.

Bacteria isolated from the vitreous samples of these patients were identified as *Granulicatella adiacens* by the 16S ribosomal RNA gene sequencing.

Conclusion: We report the first cases of post-operative endophthalmitis caused by *G. adiacens* identified by molecular genetic analysis. NVS should be considered in cases of post-operative endophthalmitis.

Keywords *Granulicatella adiacens*; Nutritionally variant streptococci (NVS); Endophthalmitis; Bleb-related infection; 16S ribosomal RNA gene; Vitrectomy

Introduction

Bacterial endophthalmitis is among the most serious infectious ocular diseases, and may result in severe visual disturbances, including blindness, enucleation, or evisceration. The causes of endophthalmitis include ocular surgery, penetrating ocular trauma, invasion from corneal or scleral abscess, and metastasis via bloodstream from systemic infectious diseases such as sepsis, catheter-related infection, liver abscess, infective endocarditis, and so on, a class of disease referred to as endogenous endophthalmitis. While most of the bacteria that cause of post-operative endophthalmitis after cataract surgery are staphylococci derived from bacterial microflora of ocular surfaces [1], the bleb-related late-onset infection after glaucoma surgery is primarily the result of streptococci followed by coagulase-negative Staphylococcus, Haemophilus influenza, and Enterococcus species [2]. To detect the causative bacteria, intraocular samples are drawn for bacterial culturing, and empiric therapy then is started. However, the culture-positive rates of intraocular tapping from post-cataract surgery and bleb-related infection remain about 63-69% and 50% respectively, and the causative bacteria in the remaining cases are unknown [2-4]. Additionally, culture-positive results often include unidentified bacteria.

Nutritionally variant streptococci (NVS) are pleomorphic Gram-variable bacteria showing fastidious growth requirements; NVS are difficult to grow on standard sheep blood agar plates, because these bacteria require supplementation with pyridoxal for growth. It has been suggested that NVS is a common cause of infectious endocarditis in cases that are negative by blood culture [5,6]. To date, 4 bacterial species have been identified as NVS: *Abiotrophia defectiva*, *Granulicatella adiacens*, *Granulicatella elegans*, and *Granulicatella balaenopterae*. Although endophthalmitis by Abiotrophia species has

been reported previously [5,7,8], endophthalmitis by Granulicatella species has not been reported except outbreak cases of postinjection endophthalmitis after intravitreal bevacizumab [9]. In this manuscript, the first report of cases of *Granulucatella adiacens* postoperative endophthalmitis, which were successfully treated by early surgeries using vitrectomy were presented.

Cases and Methods

Case 1

A 77-year-old female had complained of decreased left vision for a week. This patient had undergone repeated ocular surgeries for primary angle closure glaucoma in both eyes and post-operative bullous keratopathy in the left eye; laser iridotomy had been performed in both eyes about 17 years previously. Additionally, the patient had received trabeculectomy 12 years previously, cataract surgery 9 years previously, and Descemet stripping automated endothelial keratoplasty (DSAEK) twice (5 and 2 years previously) in her left eye. The subject had a history of hypertension and hypercholesterolemia but no immunosuppressive background. The best corrected visual acuity (BCVA) showed 20/20 in the right eye and light perception in the left eye. Intraocular pressure showed 18 mmHg in the right eye and 13 mmHg in the left eye. Slit-lamp examination revealed slight ciliary injection, corneal edema, inflammatory cells, flare, and fibrin in the anterior chamber of the left eye (Figure 1A). The corneal graft exhibited no signs of infection, including keratoprecipitate, infiltration, or endothelial plaque. An avascular filtration bleb at 11 o'clock of the left corneal limbus did not show bleb leakage or obvious blebitis (Figure 1B). The intraocular lens in the left eye was clear. The fundus of the left eye was not visible, and the B-scan ultrasonography revealed vitreous opacity. The right eye showed no signs of infection in the anterior or posterior segments. To differentiate endogenous endophthalmitis, thorough systemic examination was performed. The patient had no history of invasive procedures, including extraction of teeth, catheter insertion, intravenous drug abuse, or abdominal surgery, and body temperature was not elevated. Serologic tests revealed only a slight elevation of C-reactive protein (to 0.29 mg/dL), and white blood cell count was normal. A blood sample was subjected to bacterial culturing. Computed tomography (CT) of the chest and abdomen detected no infectious origin, including liver abscess. Echocardiography showed no evidence of infective endocarditis. Late-onset bleb-related endophthalmitis without bleb leakage was suspected because endogenous endophthalmitis was differentiated, and a vitrectomy with anterior chamber wash was performed immediately. The vitreous sample was inoculated into thioglycolate broth. During the vitrectomy surgery, the vitreous cavity was irrigated with vancomycin (VCM) 20 m/mL and ceftazidime (CAZ) 40 μg/mL. Then, topical levofloxacin (LVFX) 1.5% and cefmenoxime (CMX) 0.5% were administered six times per day each, and intravenous flomoxef was also administered empirically. Following this procedure, the BCVA of the patient's left eye recovered to 18/20 after 2 months.

The blood culture was negative (i.e., did not yield an organism). The vitreous sample was subcultured onto blood, chocolate and Sabouraud agar plates at the following day of vitrectomy, and yielded the growth of small colonies on chocolate agar plate (BY chocolate agar, BD Japan) at the day 2. Staining and microscopic examination of the organisms indicated the presence of Gram-variable pleomorphic bacteria (Figure 1C). Amplification of a portion of the 16S ribosomal RNA-encoding gene was performed using the primer pair 10F (5'-GTTTGATCCTGGCTCA-3') and 800R (5'-TACCAGGGTATCTAATCC-3'). DNA sequence analysis showed 99.7% identity with the rDNA of the *Granulicatella adiacens* type strain ATCC 49175. The novel strain was able to grow as satellite colonies around staphylococcal colonies when streaked onto a sheep blood agar plate containing staphylococci (Figure 1D). Taken together, these observations indicated that the new isolate was a strain of *G. adiacens*, and so was designated as *G. adiacens* GM01. This clinical isolate, when tested according to the CLSI M45-A2 method, exhibited no antibiotic resistance *in vitro* (Table 1).

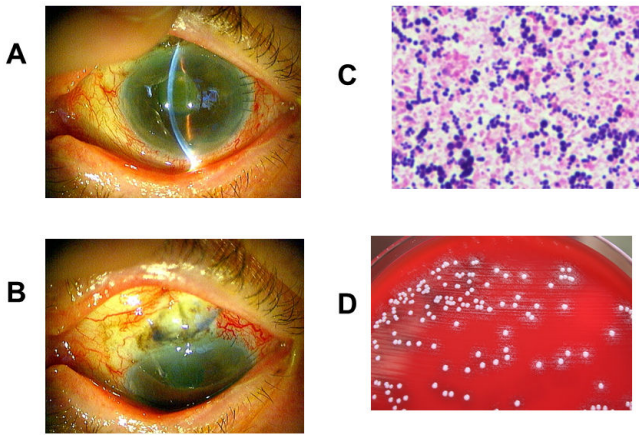

Figure 1: A) Photograph of slit-lamp examination of the left eye of Case 1 at the first visit. Observation revealed slight ciliary injection, corneal edema, inflammatory cells, flare, and fibrin in the anterior chamber. B) A downgaze-view of 1a. A filtration bleb at 11 o'clock of the left corneal limbs did not show bleb leakage. C) A micrograph of a Gram-stained specimen (x1,000) of colonies on a chocolate agar plate of the isolate from Case 1. Gram-variable pleomorphic bacteria were observed. D) The satellite phenomenon of the isolate from Case 1 growing on a sheep blood agar plate. Small colonies are observed around staphylococcal colonies.

Case 2

A 85-year-old male who had a history of hypertension and angina pectoris underwent cataract surgery and intraocular lens implantation in his left eye; no complications were reported during the operation. However, 5 days later, the subject reported symptoms of blurred vision and pain in his left eye. On the following day, this individual visited an ophthalmologist because of a further decrease of vision and was immediately referred with the suspected acute postoperative endophthalmitis on the 6th postoperative day. The BCVA was 20/20 in the right eye and light perception in the left eye. Intraocular pressure was 5 mmHg in the right eye and 9 mmHg in the left eye. Slit-lamp examination of the left eye showed ciliary injection, cells and fibrin in the anterior chamber, and hypopyon (Figure 2A). The fundus of the left eye was invisible due to vitreous opacity. B-scan ultrasonography showed dense vitreous opacity. Postoperative infectious endophthalmitis was suspected, and vitrectomy and the removal of intraocular lens was performed at the day of initial visit. Vitreous irrigation with VCM and CAZ as described above and topical LVFX and CMX (6 times per day each) and intravenous cefpirome was administered empirically. Inflammation of the left eye was improved

immediately after the vitrectomy, and the retinal tissue was not injured. However, the final BCVA of the left eye remained 20/125 after 3 months because of the pre-existing foveal scar.

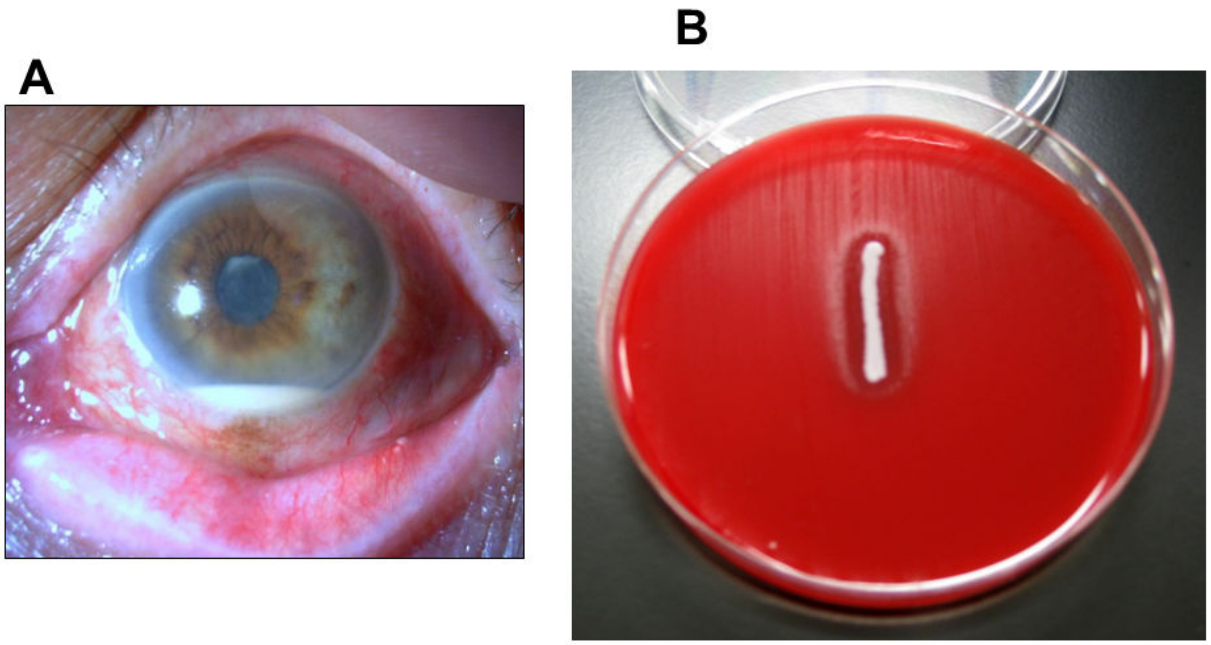

Figure 2: A) Photograph of slit-lamp examination of the left eye of Case 2 at the first visit. Ciliary injection, inflammatory cells and fibrin in the anterior chamber, and hypopyon are observed. B) The satellite phenomenon of the isolate from Case 2 growing on a sheep blood agar plate. Small colonies are observed around a streak of staphylococci.

The vitreous sample collected at vitrectomy was cultured for bacteria as described in Case 1 section, which resulted in the growth of small bacterial colonies on a chocolate agar plate in 2 days. The bacteria were able to grow as satellite colonies around staphylococcal colonies when streaked onto a sheep blood agar plate containing staphylococci (Figure 2B). DNA sequence analysis of 16S rRNA using the primer pair 8UA (5'-AGAGTTTGATCCTGGCTCAG-3') and 1485B (5'-TACGGTTACCTTACGAC-3') [10] showed 99.7% identity with that of the type strain ATCC 49175, and the strain was designated *G. adiacens* GF02. Antibiotic susceptibility testing showed increased MIC of levofloxacin (LVFX) *in vitro* (Table 1).

	MIC (μg/mL)	
Antibiotics [a]	Case 1	Case 2
PCG	≤ 0.06	≤ 0.06
ABPC	≤ 0.06	≤ 0.25
CVA/AMPC	≤ 0.03	≤ 1
CTM	NA	≤ 0.5
CTRX	NA	1
CCL	≤ 0.03	NA
CFPM	NA	≤ 0.5
IPM/CS	≤ 0.06	≤ 0.12
EM	≤ 0.06	≤ 0.12
MINO	≤ 0.06	≤ 2
LVFX	0.5	>4
CLDM	≤ 0.06	NA
ST	NA	≤ 0.25
VCM	1	NA

[a]Abbreviations: PCG: Penicillin G; ABPC: Ampicillin; CVA/AMPC: Clavulanate/Amoxicillin; CTM: Cefotiam; CTRX: Ceftriaxone; CCL: Cefaclor; CFPM: Cefepime; IPM/CS: Imipenem/Cilastatin; EM: Erythromycin; MINO: Minocycline; LVFX: Levofloxacin; CLDM: Clindamycin; ST: Sulfamethoxazole-Trimethoprim; VCM: Vancomycin

Table 1: Antibiotic susceptibilities of Granulicatella adiacens strains isolated in this study based on the CLSI M45-A2 method.

Discussion

Nutritionally variant streptococci (NVS) originally were described by Frenkel and Hirsch as streptococci that were able to grow as small satellite colonies when placed near other bacterial colonies [11]. Notably, NVS cannot grow on standard trypticase soy agar with 5% sheep blood because of their fastidious growth requirements. However, helper organisms like staphylococci, streptococci (excepting *Streptococcus pyogenes*), Enterobacteriaceae, and yeasts support the growth of NVS by producing pyridoxal or L-cysteine [11]. NVS usually

grow on chocolate agar, Brucella agar with 5% horse blood, media supplemented with pyridoxal hydrochloride 0.001%, and in thioglycolate broth [6,12,13]. In the cases described here, vitreous samples were first cultivated in thioglycolate broth and then subcultured onto chocolate agar, a method that facilitated the effective isolation of NVS from vitreous samples.

To date, NVS have been classified as strains of one of 4 bacterial species: *Abiotrophia defectiva*, *Graulicatella adiacens*, *Granulicatella elegans*, or *Granulicatella balanenopterae* [14]. The first three of these species are normal residents of the oral cavity or upper respiratory tract, but *G. balanenopterae* is not a human commensal [15]. NVS are important human pathogens of infectious endocarditis [16-19], especially in cases that test negative by blood culture [13]. In addition, NVS have also (rarely) been identified as the causative agent of ocular infections, including conjunctivitis [20], keratitis and corneal ulcers [21], infectious crystalline keratopathy [22,23], post-traumatic orbital abscess [24], chronic dacryocyctitis [25], and postoperative endophthalmitis after cataract or glaucoma surgery [5-8,26]. NVS that have previously been reported as causative agents of bacterial endophthalmitis have been limited to *Abiotrophia adiacens* (the former name of *Granulicatella adiacens*) as identified by API 20 Strep (bio-Merieux) [5], *Granulicatella adiacens* (not detailed to the methodology of species identification) [9] and *Abiotrophia defectiva* [5-8, 26]. The cases described here are the first two examples in which the causative organism was assigned as *G. adiacens* based on molecular evidence (16S rRNA gene sequencing) since the reclassification of *A. adiacens* into the genus *Granulicatella* [14].

Case 1 developed endophthalmitis long after the last intraocular surgery; 2 possible routes of the entry of the microbe were considered, either via filtration bleb or via bloodstream metastasis (derived from other infectious foci). Despite thorough examination, no evidence of endogenous endophthalmitis was detected, and this patient exhibited no history of immunodeficiency, no general infectious signs in blood test including blood culture, and no infection focus. Thus, a diagnosis of late-onset endophthalmitis without bleb leakage after a filtration surgery for glaucoma was made by exclusion. In contrast, Case 2 presented as a case of acute postoperative endophthalmitis after cataract surgery.

As described above, postoperative endophthalmitis, especially late-onset cases, frequently yield negative results on culturing. Bleb-related infections often are caused by oral bacteria like viridans-group streptococci. This case report implies the possibility that some cases of culture-negative endophthalmitis are caused by NVS. To improve the culture-positive rate, it is suggested that vitreous samples should be cultured on media (such as thioglycolate broth or chocolate agar) enriched for the growth of NVS.

Conclusion

The first case of post-operative endophthalmitis caused by *G. adiacens* in which the identity of the infecting bacteria was demonstrated by molecular genetic analysis was reported. These cases show that post-operative endophthalmitis can be caused by NVS. Therefore, it is proposed that that cultural media (such as thioglycolate broth or chocolate agar plates) that can enrich for NVS should be incorporated into screens for causative agents of endophthalmitis. NVS should be considered in cases of post-operative endophthalmitis.

Nucleotide sequence accession numbers

The nucleotide sequences of the 16S ribosomal RNA genes of the strains GM01 and GF02 are available in the DDBJ/EMBL/GenBank databases under accession nos. LC125190 and LC125191, respectively.

Patient consent

The patients described in the manuscript provided consent for publication of this case report.

Acknowledgments

We received no external financial support for the work presented in this manuscript.

Conflict of Interests

None of the authors have any competing interests to declare.

References

1. Speaker MG, Milch FA, Shah MK, Eisner W, Kreiswirth BN (1991) Role of external bacterial flora in the pathogenesis of acute postoperative endophthalmitis. Ophthalmology 98: 639-49.
2. Yamamoto T, Kuwayama Y, Kano K, Sawada A, Shoji N, et al. Study Group for the Japan Glaucoma Society Survey of Bleb-related Infection (2013) Clinical features of bleb-related infection: a 5-year survey in Japan. Acta Ophthalmol 91: 619-624.
3. Holland EJ, McDonald MB, Parekh JG, Sheppard JD (2014) Antibiotic resistance in acute postoperative endophthalmitis. Ophthalmology 121: S1-9.
4. Simunovic MP, Rush RB, Hunyor AP, Chang AA (2012) Endophthalmitis following intravitreal injection versus endophthalmitis following cataract surgery: clinical features, causative organisms and post-treatment outcomes. Br J Ophthalmol 96: 862-866.
5. Namdari H, Kintner K, Jackson BA, Namdari S, Hughes JL, et al. (1999) Abiotrophia species as a cause of endophthalmitis following cataract extraction. J Clin Microbiol 37: 1564-1566.
6. Reimer LG, Reller LB (1981) Growth of nutritionally variant streptococci on common laboratory and 10 commercial blood culture media. J Clin Microbiol 14: 329-332.
7. Hugo Lee MH, Lawlor M, Lee AJ (2015) Abiotrophia defectiva bleb-associated endophthalmitis confirmed with 16s ribosomal RNA sequencing. J Glaucoma 24: 87-88.
8. Esteban J, Montero-Sanchez R, Ortiz A, Yanez F (2005) Postoperative endophthalmitis due to Abiotrophia defectiva. Enferm Infecc Microbiol Clin 23: 455.
9. Edison LS, Dishman HO, Tobin-D'Angelo MJ, Allen CR, Guh AY, et al. (2015) Endophthalmitis outbreak associated with repackaged bevacizumab. Emerging infectious diseases 21: 171-173.
10. Finegold SM, Molitoris D, Song Y, Liu C, Vaisanen ML, et al. (2002) Gastrointestinal microflora studies in late-onset autism. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America 35: S6-s16.
11. Frenkel A, Hirsch W (1961) Spontaneous development of L forms of streptococci requiring secretions of other bacteria or sulphydryl compounds for normal growth. Nature 191: 728-730.
12. Christensen JJ, Ruoff KL (2015) Aerococcus, Abiotrophia, and Other Aerobic Catalase-Negative, Gram-Positive Cocci. Manual of Clinical Microbiology. ASM Press, Washington, DC.
13. Ruoff KL (1991) Nutritionally variant streptococci. See comment in PubMed Commons below Clin Microbiol Rev 4: 184-190.
14. Collins MD, Lawson PA (2000) The genus Abiotrophia (Kawamura et al.) is not monophyletic: proposal of Granulicatella gen. nov., Granulicatella

adiacens comb. nov., Granulicatella elegans comb. nov. and Granulicatella balaenopterae comb. nov. International journal of systematic and evolutionary microbiology 50: 365-369.

15. Lawson PA, Foster G, Falsen E, Sjoden B, Collins MD (1999) Abiotrophia balaenopterae sp. nov., isolated from the minke whale (Balaenoptera acutorostrata). Int J Syst Bacteriol 49 2: 503-506.
16. Padmaja K, Lakshmi V, Subramanian S, Neeraja M, Krishna SR, et al. (2014) Infective endocarditis due to Granulicatella adiacens: a case report and review. J Infect Dev Ctries 8: 548-550.
17. Shailaja TS, Sathiavathy KA, Unni G (2013) Infective endocarditis caused by Granulicatella adiacens. Indian Heart J 65: 447-449.
18. Jeng A, Chen J, Katsivas T (2005) Prosthetic valve endocarditis from Granulicatella adiacens (nutritionally variant streptococci). J Infect 51: e125-129.
19. Al-Tawfiq JA, Kiwan G, Murrar H (2007) Granulicatella elegans native valve infective endocarditis: case report and review. Diagn Microbiol Infect Dis 57: 439-441.
20. Barrios H, Bump CM (1986) Conjunctivitis caused by a nutritionally variant streptococcus. J Clin Microbiol 23: 379-380.
21. Christensen JJ, Facklam RR (2001) Granulicatella and Abiotrophia species from human clinical specimens. J Clin Microbiol 39: 3520-3523.
22. Paulus YM, Cockerham GC (2013) Abiotrophia defectiva causing infectious crystalline keratopathy and corneal ulcer after penetrating keratoplasty: a case report. J Ophthalmic Inflamm Infect 3: 20.
23. Ormerod LD, Ruoff KL, Meisler DM, Wasson PJ, Kintner JC, et al. (1991) Infectious crystalline keratopathy. Role of nutritionally variant streptococci and other bacterial factors. Ophthalmology 98: 159-169.
24. Teo L, Looi A, Seah LL (2011) An unusual causative agent for an orbital abscess: Granulicatella Adiacens. Orbit (Amsterdam, Netherlands) 30: 162-164.
25. Ku CA, Forcina B, LaSala PR, Nguyen J (2015) Granulicatella adiacens, an unusual causative agent in chronic dacryocystitis. See comment in PubMed Commons below J Ophthalmic Inflamm Infect 5: 12.
26. Horstkotte MA, Dobinsky S, Rohde H, Knobloch JK, Hassenstein A, et al. (2010) Abiotrophia defectiva endophthalmitis with retinal involvement and infiltrative keratitis: case report and review of the literature. Eur J Clin Microbiol Infect Dis 29: 727-731.