Spectrum of post-keratoplasty ocular infection with treatment outcome at a tertiary centre in North India

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Abstract:

Aim: To report the microbiological spectrum with their antimicrobial resistance and prognosis in post-keratoplasty [Penetrating Keratoplasty (PK), Deep anterior lamellar Keratoplasty (DALK), and Descemet Stripping Endothelial Keratoplasty (DSEK)] infection at a tertiary care centre in North India.

Material and methods: A retrospective analysis of 106 keratoplasties was performed from 2007 to 2012. 86 eyes underwent PK, 8 eyes DALK and 12 DSEK. A detailed microbiological work up including Gram staining, 10% KOH wet mount, culture on blood agar and Sabouraud's Dextrose Agar, was done in patients with post-keratoplasty infections.

Results: 8 (7.54%) eyes (PK–7, DSEK–1, DALK–0) developed corneal infection. In two eyes (including one that underwent DSEK) *Pseudomonas aeruginosa was isolated.* Both *Pseudomonas* were resistant to all anti-microbial except Polymyxin B. In two patients *Streptococcus pneumoniae* was isolated which were sensitive to commonly used antibiotics. One patient developed *Candida albicans* which showed resistance to all commonly used anti-fungals (CLSI-44A), except Amphoterecin B. One isolate each of *Staphylococcus aureus, Proteus vulgaris and Acinetobacter baumanii* was identified in 3 different patients, which were all susceptible to common antibiotics. All patients except one (*P.aeruginosa*) responded well to susceptible drugs.

Conclusion: High infection rate in post-keratoplasty patients with great diversity of microorganism and increased microbial resistance necessitates detailed microbiological work up in each case.

Key words: Post-keratoplasty, Infection, Candida, Pseudomonas, DSEK, MDR

Introduction:

Post-keratoplasty infection is common but devastating complication associated with ocular morbidity and poor visual outcome.[1,2,3,4] Infection in patients who had undergone keratoplasty can be either due to poor host defense, direct dissemination of microbes from donor to recipient, as MK media can itself act as a good culture media or due to absence of corneal nerves in donor cornea, ocular surface problems, poor epithelialization, limbal stem cell deficiency, suture related problems and post-operative long use of steroids all contribute to poor host defense.[2,5,6,7] Steroid instillation used to prevent graft rejection increases the chances of microbiological invasion especially fungi.

Aim:

The purpose of this study is to report the microbiological spectrum with their antimicrobial resistance and prognosis in post-keratoplasty infection at a tertiary care centre in North India.

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Materials and Methods:

A retrospective analysis of 106 eyes of 102 patients who underwent corneal transplant [Penetrating Keratoplasty (PK), Deep anterior lamellar Keratoplasty (DALK), and Descemet Stripping Endothelial Keratoplasty (DSEK)] from 2007 to 2012 was performed. 86 eyes underwent PK, 8 eyes DALK and 12 eyes underwent DSEK surgery.

Patients who developed corneal infiltrate within 6 months were only included in this study. All such patients were scraped by No. 15 Bard Parker blade. Direct microscopic examination using Gram's stain and 10% KOH wet mount was performed in all patients. The scraped material was further inoculated on blood agar and Sabouraud's dextrose agar. Blood agar and Sabouraud's dextrose agar were incubated at 37°C and 25°C respectively in BOD incubator. Growth was examined by Gram's stain and biochemical tests including oxidase and catalase tests. The growth was confirmed as *Candida* spp. by germ tube formation, ability to growth at 42°C and sugar assimilation with growth on CHROMagar. Antimicrobial susceptibility was performed with Kirby Bauer method for bacteria and antifungal susceptibility testing with CLSI44A. Antibiogram was done against Ampicillin (10µg), Carbenecillin (10µg), Ceftriaxone (30µg), Gentamicin(10µg), Ciprofloxacin(5µg), Levofloxacin(5µg), amikacin(30µg), Imipenem(10µg) and Polymyxin B(300units). For fungi, antibiogram was performed with fluconazole (25µg), itrconazole (10µg), voriconazole (1µg) and amphoterecin B (100units).

RESULTS:

Out of the total 106 keratoplasties that were performed, 8 (7.54%) eyes of 8 patients developed post-keratoplasty infection (PK–7, DSEK–1, DALK–0) .Figure1A,B) Four patients were female and 4 were male. Mean age of affected patients were 44.28yrs. Most common etiology for performing keratoplasty was corneal scarring secondary to corneal infection followed by pseudophakic bullous keratopathy. 4 cases presented with pain and lacrimation in affected eye within 24 hrs of keratoplasty. One patient presented on 3rd day of keratoplasty. Two cases presented at 7th and 15th day and one presented after 1 month following keratoplasty (Table 1).

On Gram's stain, there were pus cell revealed along with yeast cell in one and gram negative cocco-bacilli in another smear. (Figure2A,B) There was growth on blood agar and Sabourauds Dextrose Agar. (Figure3A,B) *Pseudomonas aeruginosa* was the most common organism isolated from affected eye within 24 hours of keratoplasty. Both cases of *Pseudomonas* and one each case of *Acinetobacter baumanii* and *Staphylococcus aureus* were isolated in patients who developed infiltrate within 24 hrs of keratoplasty. *Candida albicans* was isolated from a female patient who complained of gritty sensation after 3 days following corneal transplant. *Streptococcus pneumoniae* was isolated from two patients with pain and discharge after 7th and 10th day of keratoplasty respectively. *Proteus vulgaris* was isolated from a 17 year old girl who presented with pain and discharge after 1 month of keratoplasty (Table 1).

On Kirby Bauer disk diffusion method, both isolated *Pseudomonas aeruginosa* showed susceptibility only to polymyxin B, being resistant to piperacillin, gentamicin, ceftazidime, amikacin, ciprofloxacin, imipenem and meropenem.Figure4 Isolated *Acinetobacter baumanii* and *Proteus vulgaris* were susceptible to piperacillin, gentamicin, ceftazidime, ciprofloxacin, imipenem, meropenem and polymyxin B

Streptococcus pneumoniae was susceptible to penicillin, ciprofloxacin, gentamicin and vancomycin. Isolated *Stapylococcus aureus* showed susceptibility to ciprofloxacin, gentamicin and vancomycin and showed resistant to penicillin. *Candida albicans* was only susceptible to amphoterecin B being resistant to azoles as demonstrated by CLSI44A guidelines (Table 2).

Antimicrobial drops were instilled in affected eye corresponding to their antibiogram. All the patients except one had good response with reduction in corneal infiltrates. One patient infected with *Pseudomonas aeruginosa* who underwent DSEK, failed to have any response and developed endophthalmitis after 24 hours despite continuous topical moxifloxacin instillation. The sensitivity report which was received after 48 hours showed that the organism was only sensitive to Polymyxin B.

Out of 8 cases of infectious keratitis, 3 patients were infected with mulit-drug resistant microorganisms that were resistant to commonly used antimicrobials. In 7 (87.5%) eyes including two eyes infected with multidrug resistant organism, there was complete resolution of infiltrates with good clinical outcome.

Discussion:

Infection after keratoplasty is a setback for patients with poor treatment outcome usually. Meticulous microbiological examination with intense antimicrobial therapy and timely monitoring is necessary to achieve good final visual outcome in graft infection. In this era of multi-drug resistance organism, microbiological profile and sensitivity pattern can only predict the exact nature of infection and the correct treatment required for the particular case. Every micro-organism has a varied spectrum thus should be dealt differently. Gram-positive cocci including *Staphylococcus aureus, Streptococcus pneumoniae, Coagulase negative Staphylococci* are common causative agent whereas among Gram-negative bacteria, *Pseudomonas aeruginosa* is commonly isolated.[8,9]

In our study, incidence of post-keratoplasty infection is higher compared to most studies. Malathi Ambulingam reported that incidence of postoperative endophthalmitis in Tamilnadu was only 0.5% (10/1949) as only ten PK surgeries developed infections[10]. *Enterococcus fecalis* (3) was most commonly isolated microorganism followed by *Pseudomonas aeruginosa* (2) and one case each of Methicillin resistant *Staphylococcus aureus, Alkaligenes fecalis, Kleibsella pneumoniae, Pseudomonas stutzeri* and *Aspergillus flavus*. Low post-keratoplasty infection was also shown by Kattam HM et al (0.11%),[11] Taban M (0.382%)[12] and Thomas M Aaberg (0.178%).[13] In our study, Improper follow-up was the most common cause of high incidence of post-keratoplasty infection as 5 patients developed suture infiltrate after hospital discharge. Long storage of the cornea in MK media was another risk factor. However, a study by Wakimasu et al., reported similar post-keratoplasty infection compared to our study. In his study 27 patients out of 253 developed microbial keratitis (14 bacterial and 13 fungal).[14] Seven eyes were infected with Methicillin resistant *Staphylococcus aureus* & *Methicillin Resistant Staphylococcus Epidermidis.* Candida infection was present in 8 eyes.

Spectrum of the isolated microorganisms varied largely in our study, as Gram-positive bacteria, Gram-negative bacteria and fungus (candida) were isolated. In contrast to the study by RB Vajpayee et al. who reported that Gram positive cocci (*Staphylococcus epidermidis,* 55.8%) being the most common cause of post-keratoplasty infection followed by *Staphylococcus aureus, Acinetobacter* spp., *Pseudomonas*

aeruginosa, Aspergillus fumigatus, *Streptococcus pneumoniae* and *Fusarium solani*[3], our study showed that Gram negative bacilli (two *Pseudomonas aeruginosa* and one each *Proteus vulgaris* & *Acinetobacter bowmanii*) had higher incidence of post-keratoplasty infections. Gram positive cocci (two *Streptococcus pneumniae* and one *Staphylococcus aureus*) were common occurrence in post-keratoplasty infections. One case of *Candida albicans was also identified from suture infiltrate*. Wagoner MD reported *Streptococcus pneumoniae* as the most common cause of post-keratoplasty infections in children.[15]

In our study, both isolated *Pseudomonas aeruginosa* were susceptible only to polymyxin B with resistant to other drugs. Michael S Insler and his team reported a case of post-keratoplasty endophthalmitis caused by *Pseudomonas aeruginosa* showing resistance to gentamicin.[16] Ana Paula *et al.*, reported two cases of MDR *Pseudomonas aeruginosa* infection after cornea transplant. These isolated *Pseudomonas aeruginosa* showed absence of response to intravenous ceftazidime and imipenem eye drop (50 mg/ml).[17] A. Panda reported a case series of 7 eyes infected with multidrug resistant *Pseudomonas aeruginosa*. All isolates were susceptible only to polymyxin B. All the corneo-scleral rims were preserved in MK media. She suggested that although MK media already contains Gentamicin, *Pseudomonas aeruginosa* resistant to Gentamicin, could easily thrive in the media.[18] Insler et al reported that the emergence of more antibiotic resistant micro-organisms in antibiotic supplemented media may result in donor to host contamination following keratoplasty. Increased length of storage is a major cause of transmission.[16] In our study also, both the cases of Pseudomonas were only susceptible to Polymixin B.

Pseudomonas aeruginosa is a potential contaminant of pharmaceutical and cosmetic preparation and is a common hospital acquired (nosocomial) pathogen. The nosocomial microorganism is usually highly resistant to most of the available antibiotics, giving very limited options to the Ophthalmologists for use of antibiotics.[19] *Streptococcus pneumoniae* was another common causative agent of post-keratoplasty corneal infection. This pathogen, being commensal in throat may reach the ocular surface through nasolacrimal duct and cause corneal infection. Moore PJ reported *Streptococcus pneumoniae* endophthalmitis following corneal transplant.[20] In our study there was one case of infectious keratitis following Descemet Stripping Endothelial Keratoplasty (DSEK) surgery. Hannus SB had earlier also reported three cases of infectious keratitis after DSEK surgery. These cases of post-DSEK infections were caused by *Pseudomonas aeruginosa, Streptococcus pneumoniae* and *Enterococcus faecalis*.[21]

Candida albicans, a yeast like fungi may also cause keratitis in patients who have undergone keratoplasty. MR Sedaghat reported a case of *Candida albicans* interface infection after deep anterior lamellar keratoplasty in an 18 year old female presenting with keratoconus. Keratitis was completely resolved after 10 days of continuous interface irrigation with amphoterecin B.[22] Koenig SB reported a case of Candida keratitis after descemet stripping automated endothelial keratoplasty (DSAEK) in a 90 year old male with pseudophakic bullous keratopathy. Despite intensive treatment, patient failed to respond and enucleation was done.[23]

Acinetobacter baumanii is a gram negative bacillus that causes nosocomial infection. Kaun Jen Chen et al (2008) reported a case of post-keratoplasty endophthalmitis caused by *Acinetobacter*.[24] *Proteus vulgaris* was isolated in a 17 year old girl in our case series after one month of the corneal transplant. Lam DS et al (1998) reported a case of post-keratoplasty endhophthalmitis caused by *Proteus mirabilis* in a diabetic patient. Isolated *Proteus mirabilis* was resistant to gentamicin.[25]

Corneo-scleral rim is a major source of microbes. Kehyani K et al. reported that 13% of the corneo-scleral rims had microbes including fungi in 28 eyes. All fungi were *Candida* species on culture. They reported that post-keratoplasty fungal infections occurred only in those cases in which contaminated cornea was transplanted.[26]

Conclusion:

Post-keratoplasty infection is an infrequent complication of corneal transplantation. Reduced corneal sensation with frequent instillation of corticosteroid eye drops enhances the chances of post keratoplasty infections. There is also a great risk of donor to host transmission. Huge diversity of microorganism and emergence of resistance to antimicrobials necessitates the ophthalmologist to scrape the cornea in each patient with corneal infiltrate and subject to antibiotic susceptibility, so that the correct organism along with resistance to drugs be established and the devastating sequel like complete vision loss or painful blind eye can be prevented. This will help in ensuring good clinical outcome.

References:

- Bates AK, Kirkness CM, Ficker LA, Steele AD, Rice NS. Microbial keratitis after penetrating keratoplasty. Eye (Lond). 1990;4 (Pt 1):74-8
- Fong LP, Ormerod LD, Kenyon KR, Foster CS. Microbial keratitis complicating penetrating keratoplasty. Ophthalmology. 1988 Sep;95(9):1269-75
- Vajpayee RB, Sharma N, Sinha R, Agarwal T, Singhvi A. Infectious keratitis following keratoplasty. Surv Ophthalmol. 2007 Jan-Feb;52(1):1-12
- Wright TM, Afshari NA. Microbial keratitis following corneal transplantation. Am J Ophthalmol. 2006 Dec;142(6):1061-2
- Leahey AB, Avery RL, Gottsch JD, Mallette RA, Stark WJ. Suture abscesses after penetrating keratoplasty. Cornea. 1993 Nov;12(6):489-92
- Wagoner MD, Al-Swailem SA, Sutphin JE, Zimmerman MB. Bacterial keratitis after penetrating keratoplasty: incidence, microbiological profile, graft survival, and visual outcome. Ophthalmology. 2007 Jun;114(6):1073-9
- Das S, Constantinou M, Ong T, Taylor HR. Microbial keratitis following corneal transplantation. Clin Experiment Ophthalmol. 2007 Jul;35(5):427-31
- Akova YA, Onat M, Koc F, Nurozler A, Duman S. Microbial keratitis following penetrating keratoplasty. Ophthalmic Surg Lasers. 1999 Jun;30(6):449-55
- Al-Hazzaa SA, Tabbara KF. Bacterial keratitis after penetrating keratoplasty. Ophthalmology. 1988 Nov;95(11):1504-8
- Jambulingam M, Parameswaran SK, Lysa S, Selvaraj M, Madhavan HN. A study on the incidence, microbiological analysis and investigations on the source of infection of postoperative infectious endophthalmitis in a tertiary care ophthalmic hospital: an 8-year study. Indian J Ophthalmol. 2010 Jul-Aug;58(4):297-302
- Kattan HM, Flynn HW Jr, Pflugfelder SC, Robertson C, Forster RK. Nosocomial endophthalmitis survey. Current incidence of infection after intraocular surgery. Ophthalmology. 1991 Feb;98(2):227-38
- Taban M, Behrens A, Newcomb RL, Nobe MY, McDonnell PJ. Incidence of acute endophthalmitis following penetrating keratoplasty: a systematic review. Arch Ophthalmol. 2005 May;123(5):605-9
- Aaberg TM Jr, Flynn HW Jr, Schiffman J, Newton J. Nosocomial acute-onset postoperative endophthalmitis survey. A 10-year review of incidence and outcomes. Ophthalmology. 1998 Jun;105(6):1004-10
- Makimasu K, Sotozono C, Shimizu Y, Inatomi T, Sano Y, Nishida K, Yokoi N, Kinoshita S. [A retrospective analysis of infection after corneal transplantation]. Nihon Ganka Gakkai Zasshi. 2004 Jun;108(6):354-8.
- Wagoner MD, Al-Ghamdi AH, Al-Rajhi AA. bacterial keratitis after primary pediatric penetrating keratoplasty. Am J Ophthalmol 2007;143 (6):1045-7

- Insler MS, Cavanagh HD, Wilson LA. Gentamicin resistant pseudomonas endophthalmitis after penetrating keratoplasty. Br J Ophthalmol 1985;69:189–91
- Ana Paula Miyagusko Taba Oguido, Antonio Marcelo Barbante Casella, Ana Luisa Hofling-Lima, Sergio Arruda Pacheco, Paulo José Martins Bispo, Fernanda Marques. *Pseudomonas aeruginosa* Endophthalmitis after Penetrating Keratoplasty Transmitted from the Same Donor to Two Recipients Confirmed by Pulsed-Field Gel Electrophoresis. J Med Microbiol 2011;49 (9):3346-3347
- Panda A, Satpathy G, Sethi HS. Survival of Pseudomonas aeruginosa in M-K preserved corneas. Br J Ophthalmol. 2005 Jun;89(6):679-83
- Ritterband, D. C., et al. 2006. Efficacy and safety of moxifloxacin as an additive in Optisol-GS a preservation medium for corneal donor tissue. Cornea 25:1084–1089
- Moore PJ, Linnemann CC, Sanitato JJ edt al. Pneumococcal endophthalmitis after corneal transplantation: control by modification of harvesting techniques. Infect Contro Hosp Epidemiol. 1989 Mar;10(3):102-5
- Hannus SB, Chew HF, Eagle RC. Late onset infectious keratitis after descemet stripping endothelial keratoplasty with vent incision. Cornea 2011; 30 (2):229-32
- Mohammad R Sedaghat, SS Hosseinpoor . Candida albicans interface infection after deep anterior lamellar keratoplasty. Indian J Ophthalmol 2012:60(4):328-30
- Koenig SB, Wirostko WJ, Fish RI, Vovert DJ. Candida keratitis after descemet stripping and automated endothelial keratoplasty. Cornea 2009:28(4):471-3
- KJ Chen, CH Hou, CC Lai et al. endophthalmitis caused by Acinetobacter baumanii: report of two cases. J Clin Microbiol 2008;46(3):1148-1150
- Lam DS, Kwok AK, Chew S. Post keratoplasty endophthalmitis caused by Proteus mirabilis. Eye (Lond) 1998;12:139-40
- Keyhani K, Seedor JA, Shah MK, Terraciano AJ, Ritterband DC. The incidence of fungal keratitis and endophthalmitis following penetrating keratoplasty. Cornea. 2005 Apr;24(3):288-91

Table1: Shows demographic data with isolation of microorganisms

Case no	Age	Sex	Duration	Microorganism isolated
1	35	M	24 hrs	*Pseudomonas aeruginosa*
2	53	F	3 days	*Candida albicans*
3	44	M	24 hours	*Staphylococcus aureus*
4	56	M	7 days	*Streptococcus pneumoniae*
5	17	F	1 month	*Proteus vulgaris*
6	62	F	24hrs	*Pseudomonas aeruginosa*
7	39	F	24 hrs	*Acinetobacter baumanii*
8	48	M	15 days	*Streptococcus pneumoniae*

Table 2: showing isolated microorganism with their antibiogram and treatment response

Case no.	Isolated organism	Susceptibility	Resistant	Treatment given	Response	Surgical treatment
1	*Pseudomonas aeruginosa*	Polymyxin B	Carbenecillin, Gentamycin, Ceftazidime, Amikacin, Imipenem Levofloxacin	Polymyxin B	Cured	No need
2	*Candida albicans*	Amphoterecin B	Azole Resistant	Amphoterecin B	Cured	No need
3	*Staphylococcus aureus*	Ciprofloxacin, Vancomycin, Gentamycin	Penicillin	Vancomycin	cured	No need
4	*Streptococcus pneumoniae*	Penicillin, Gentamycin, Levofloxacin, Vancomycin	No	Vancomycin	cured	No need
5	*Proteus vulgaris*	Ampicillin, Gentamycin, Ceftazidine, Levofloxacin, Imipenem	No	Moxifloxacin	Cured	No need
6	*Pseudomonas aeruginosa*	Polymyxin B Imipenem (Intermediate sensitive)	Carbenecillin, Gentamycin, Ceftazidime, Amikacin, Levofloxacin, Imipenem	Polymyxin B	No response	Evisceration
7	*Acinetobacter baumanii*	Carbenecillin, Ceftazidime, Gentamycin, Levofloxacin, Imipenem, Polymyxin B	No	Moxifloxacin	cured	No need
8	*Streptococcus pneumoniae*	Penicillin, Gentamycin, Levofloxacin, Vancomycin	No	Vancomycin	Cured	No need

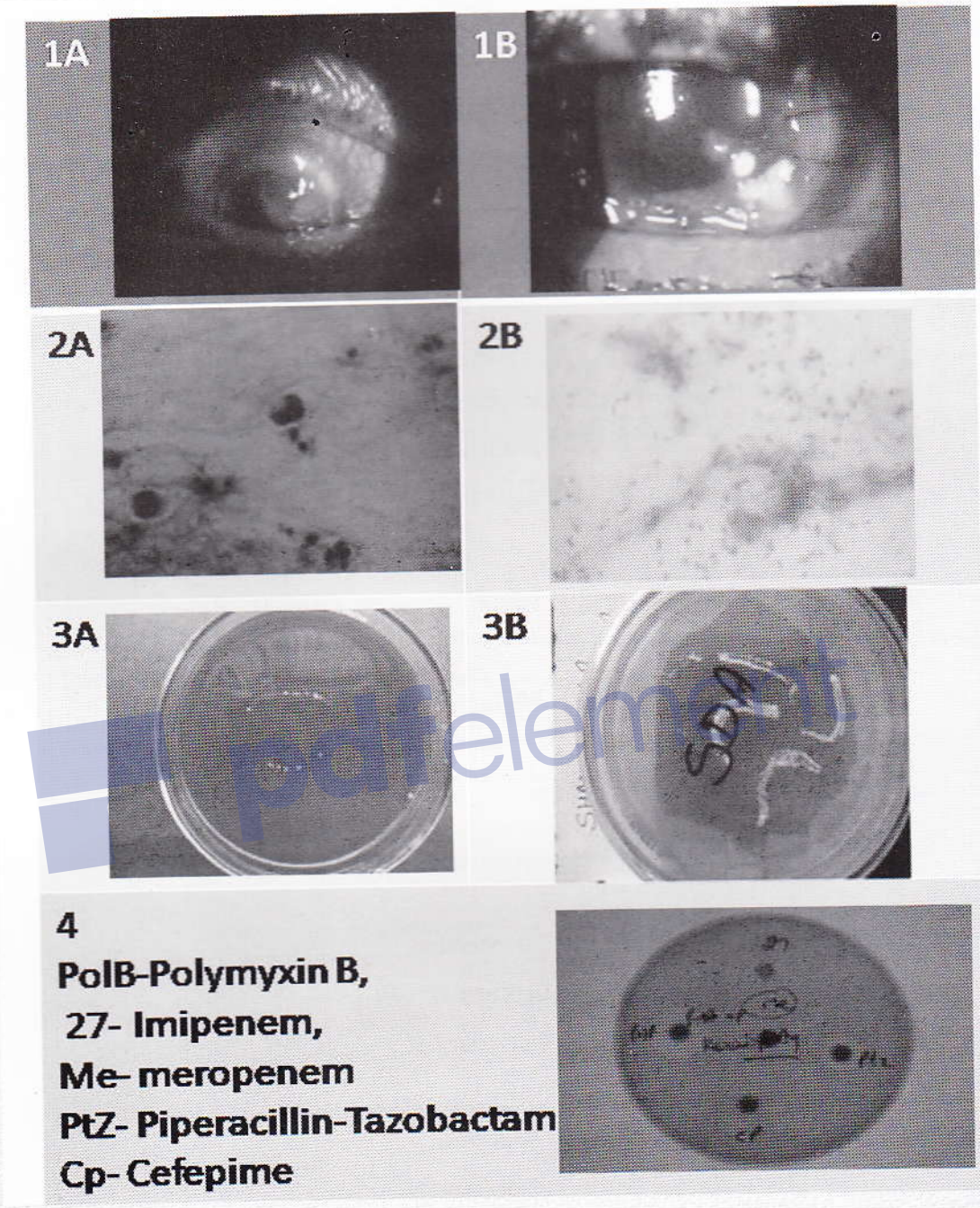

Figure 1A,1B: Clinical photographs showing *Pseudomonas aeruginosa* suppurating lesion and Candida suture infiltrates respectively

Figure 2A,2B: Gram's stain showing yeast cells and gram negative coccobacilli respectively

Figure 3A,3B: Growth on Blood agar and Sabouraud Agar respectively

Figure 4: Susceptibility of *Pseudomonas aeruginosa* to only polymyxin B on Mueller Hinton Agar