· Original article ·

Comparison of different diagnostic contact lens disinfection methods used in ophthalmic department in Chinese hospitals

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眼科诊断用接触镜的消毒现状及消毒效果比较

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摘要

目的:调查国内医院对眼科诊断用接触镜的消毒现状,评估其消毒效果。

方法:采用方便抽样的方法对10所医院进行调查,发现氯霉素滴眼液冲洗、75%乙醇搽拭和0.05%的二氯异氰尿酸钠(NaDCC)浸泡是目前我国临床最常用的眼科诊断用接触镜消毒方法。将被金黄色葡萄球菌、表皮葡萄球菌或铜绿假单胞菌污染的眼科检查用接触镜,用上述三种消毒剂按照以下方式进行消毒:1)擦拭;2)浸泡消毒5min;3)浸泡消毒10min,$n=9$. 然后镜面采样,对细菌群落培养并计数以评估细菌量,观察消毒效果。同时评估消毒后镜头的清晰度。

结果:细菌负荷量为1×10^8/mL×50μL/镜头等于5×10^6/镜头。NaDCC或乙醇消毒显著降低了细菌量;擦拭消毒时,75%的乙醇和NaDCC的消毒效果与氯霉素滴眼液比较差异显著($P\leqslant0.01$),但不能达到临床消毒要求,仍然存在交叉感染的风险。浸泡消毒效果与时间正相关,75%的乙醇和NaDCC浸泡10min最有效,能完全达到临床消毒要求,氯霉素滴眼液浸泡10min仍然不能达到临床要求。但乙醇会影响镜头的清晰度。

结论:NaDCC浸泡消毒10min或更长时间是一种简单有效的眼科诊断用接触镜消毒方法。

关键词:医院感染;氯霉素;绿脓杆菌;金黄色葡萄球菌;表皮葡萄球菌;二氯异氰尿酸钠

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Abstract

• AIM: To assess the disinfection methods of diagnostic contact lens and their efficacy in ophthalmology in China.

• METHODS: Ten Chinese hospitals were surveyed and indicated three commonly used disinfectants 0.05% sodium dichloroisocyanurate (NaDCC), 75% EtOH, or chloramphenicol eye drops (Minims®: 0.5%). Lenses were infected with *staphylococcus aureus*, *staphylococcus epidermidis*, or *pseudomonas aeruginosa*, and then disinfected by; 1) wiping; 2) immersing in disinfectant for 5min; 3) immersing in disinfectant for 10min; $n=9$. Swab wipes from the lenses were cultured for 24h and colony-forming units were counted to assess bacterial load. Lens clarity was subjectively scored for each method.

• RESULTS: The bacterial load was 1×10^8/mL ×50μL/lense equals 5×10^6/lense. Of the three methods the use of 75% EtOH and NaDCC are significantly more effective when used for wiping compared to eye drops ($P\leqslant0.01$). Wiping with NaDCC or EtOH significantly reduced bacterial load but results were variable and the threat of cross-infection is still present. They were effective if used for 10min, but EtOH adversely affected lens clarity in contrast to NaDCC which had no adverse affects on the lens. Chloramphenicol eye drops were ineffective.

• CONCLUSION: NaDCC immersion for 10min or longer appears to be a simple and effective way to disinfect diagnostic contact lenses during ophthalmic examination.

• KEYWORDS: hospital infection; chloramphenicol; dichloroisocyanurate; pseudomonas aeruginosa; staphylococcus aureus; staphylococcus epidermidis
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INTRODUCTION

Contact lenses are important for routine use in ophthalmic diagnostics and treatment, such as slit lamp examinations. In many ophthalmic examinations there is direct contact with the patients' cornea. Reuse of contact lenses poses problems of cross infection if they are not adequately disinfected[1]. Therefore, maintaining a sterile environment becomes tremendously important but has not been well addressed[2]. Improper disinfection of contact lens results nosocomial outbreak of keratoconjunctivitis at the Ophthalmology Clinic and it is not rare[3]. Other infectious diseases can also be transmitted by polluted contact lens. Previous research has shown that the Hepatitis B virus can be detected in about 1/4 of the tonometer heads used to measure the intraocular pressure of Hepatitis B virus carriers[4]. Moreover, the human immunodeficiency virus (HIV) was also found in the tears, the aqueous humor, and the subretinal fluid of acquired immunodeficiency syndrome (AIDS) patients and is a potential source of risk *via* equipment contamination[5-8]. During the severe outbreak and epidemic of acute respiratory syndrome (SARS), the Chinese Ophthalmological Society recommended rinsing contact lens under running water, and then wiping with 75% EtOH or 3% hydrogen peroxide impregnated swabs in order to reduce the possibility of cross infection caused by the contact lenses[9]. Thus, it is clear that contact lenses can be infected by some factors such as the patient's tears and ignoring this point may result in cross infection[10]. There are no quantitative studies that compare the effects and efficacy of common disinfection methods for ophthalmic contact lens[11]. Previous studies have shown that alcohol reduces the resolution of the lens, which affects the examination results, and is also harmful to the contact lenses[12]. The cost of using disposable lenses in many developing countries is unacceptably high in spite of the risk of cross infection[13]. Therefore, it is also necessary to find an effective disinfection method that does not affect the resolution of the lens and thus be of beneficial to developing countries that cannot afford disposable and expensive lenses. The purpose of this study is to examine the current disinfection methods in Chinese ophthalmic clinics and compare their efficacies.

MATERIALS AND METHODS

Survey of Hospital Practices and Contact Lenses Used A survey was conducted in 10 Chinese hospitals (nine 3rd-grade class A public hospitals and one private hospital). Ten representatives from the hospitals responded the survey (feedback rate was 100%), which includes one director of an ophthalmology department, five head nurses and four attending physicians. The survey contained information as to the grade of the hospital, the name of the contact lens used for examination, the name and the concentration of the disinfectant, and disinfection methodology as well. On the basis of the hospital survey (see results section) three general disinfectants (0.05% sodium dichloroisocyanurate (NaDCC, sodium 3,5-dichloro-2,4,6-trioxo-1,3,5-triazinan-1-ide; $C_3Cl_2N_3NaO_3$), 75% EtOH, and chloramphenicol eye drops {2,2-dichloro-N-[1,3-dihydroxy-1-(4-nitrophenyl) propan-2-yl] acetamide} were selected for comparison. The contact lenses used for this experiment were all prisms manufactured by 66 Vision-Tech Co., Ltd of Suzhou.

Bacteria Selection and Culture Methods Three kinds of common intraocular pathogenic bacteria were selected: *staphylococcus aureus* (*S. aureus*), *staphylococcus epidermidis* (*S. epidermidis*), and *pseudomonas aeruginosa* (*P. aeruginosa*), since those three bacterias were reported to be most common in ocular surface[14]. Bacteria were cultured on Columbia agar plates for 24h at 37℃[15]. Samples were collected and diluted with reagents to a final concentration of 1×10^8/mL. A 50μL aliquot of the bacterial solution was then added to the concave surface of the lens and the bacteria smeared evenly onto the lens surface. Three minutes later after the inoculation, a bacterial-free reagent swab was used to collect the sample from the lens. This was used to inoculate a Columbia blood agar plate, which was cultured for 24h and then the resultant number of colony-forming units (CFU) were counted (Figure 1) using a microscope. The disinfectants were applied to the lenses in three different ways. 1) the lens was wiped with a swab containing the disinfectant; 2) the lens was immersed in the disinfectant for 5min; 3) the lens was immersed in disinfectant for 10min. After that, a sample was collected immediately and treated by methods as described above. In some cases colony counts were too high to allow manual counting (Figure 2). In these cases, the bacteria obtained after 24h were collected in a known solution volume and a sample then serially diluted and replanted for another 24h. The number of colonies were counted and this number was multiplied by the serial dilution in order to obtain an estimation of the colony-forming units; in this way the results could be compared in the case where there were low numbers of colonies formed. Bacterial counts from lenses that were not treated with a disinfection method were very high and precluded counting CFU, in this case, bacteria were dislodged from the plates and diluted with 10mL normal saline. A 10μL sample was then diluted with 90μL normal saline, mixed well by vortexing plated on a haemocytometer plate on which the numbers of bacteria were counted. Values are given for approximate comparison with CFUs. The culture methods, strains used and sterilized methods conform the SFDA and Chinese Ophthalmology Society guidelines.

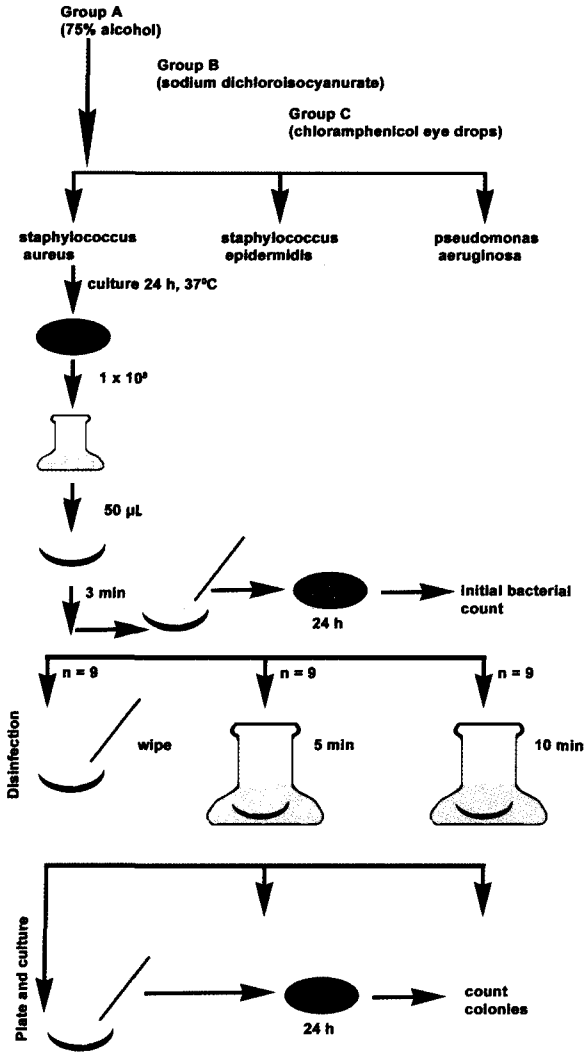

Figure 1 Schematic representation of the experimental design.

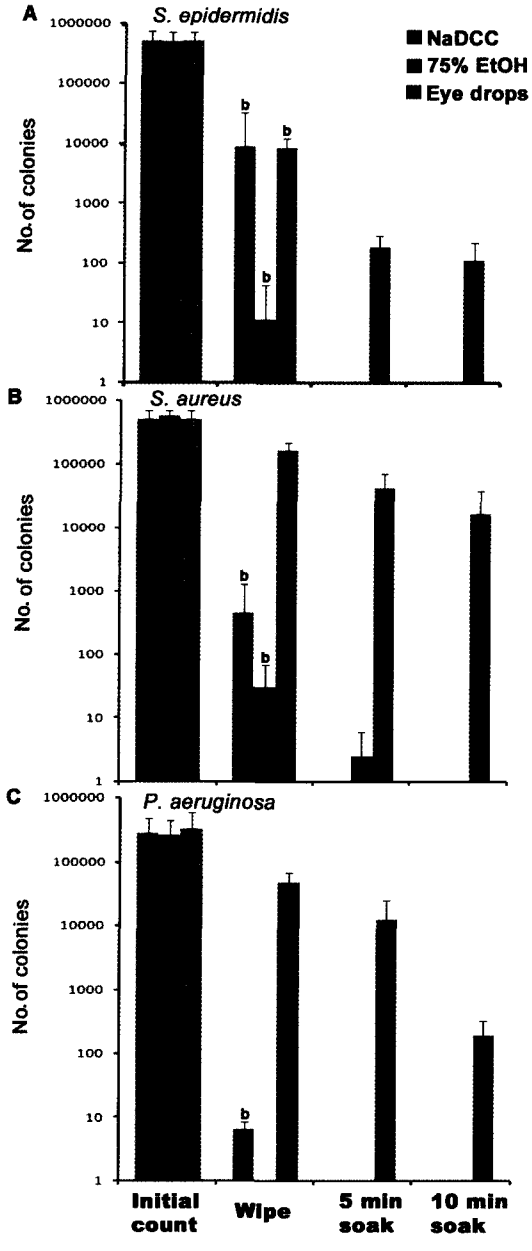

Figure 2 Comparison of the disinfection effects of NaDCC, 75% EtOH and chloramphenicol eye drops on three kinds of bacteria using three different disinfection methods In some cases the ± standard deviations were sufficiently large to preclude showing the downward bar (indicated by a). Note that value for 5min of NaDCC in B is 0. 22±0. 7 and in C for the EtOH wipe is 0. 22±0. 62 and 0. 11±0. 33 for 5min of NaDCC. Colony counts were significantly higher when the lenses were disinfected by the wiping method ($P \leqslant 0.01$ indicated by b) compared to the soaking methods when using NaDCC or 75% EtOH.

Lens Clarity Inspection Method Four doctors (attending physicians or more senior physicians) were asked to score the clarity of the contact lens at the end of each experiment (double-blind method). The lenses were graded from level 1 to 5 based on the clarity or turbidity of the lens. A score of 5 (maximum score) = clear; 4 = blurry and slightly foggy; 3 = blurry and foggy; 2 = blurry; 1 = turbid.

Statistical Analysis Each experiment was replicated nine times. The data are expressed as a mean±SD. Kruskal-Wallis test was used to compare the medians, and multiple comparisons were performed with Nemenyi tests and the P values were corrected using bonferroni method. A $P \leqslant 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS software (version 18. 0; SPSS Inc, Chicago, IL, USA).

RESULTS

Survey Results Current Disinfection Practices in a selection of Chinese Ophthalmology Units. Ten Chinese hospitals were surveyed by using a questionnaire and onsite observation. The results of the survey indicated that five hospitals (50%) used antibiotic eye drops (*e. g.* chloramphenicol, lincomycin hydrochloride eye drops), four hospitals (40%) used alcohol (two of the four hospitals used 75% EtOH, one used 95% EtOH and another one hospital used anhydrous alcohol) and one hospital (10%) used a lactic acid fumigation box to carry out disinfection.

Comparison of theThree Different Treatments It is clear from Figure 2 that wiping the lenses with NaDCC, 75% EtOH or eye drop solutions is a relatively ineffective method of disinfecting the lenses. Although the bacterial load was considerably reduced by wiping, for example in the case of *P.*

aeruginosa and the use of 75% EtOH (0.22±0.62 CFUs), there were still sufficient numbers of bacteria to warrant concern. This was particularly so in the case of *S. epidermidis* and to a lesser extent with *S. aureus*. It is also clear that soak for longer periods of time in solution can significantly decrease the bacterial load and can thus reduce the risk of cross infection ($P\leqslant0.01$). Of the three methods the use of 75% EtOH and NaDCC are significantly more effective when used for wiping compared to eye drops ($P\leqslant0.01$) except for one case, in which there was no significant difference in number of CFUs when wiping with NaDCC or chloramphenicol eye drops for *S. epidermidis*, although NaDCC was significantly more effective than the eye drops in reducing *S. aureus* or *P. aeruginosa*. A 5min soak in EtOH was generally effective for removing *S. epidermidis* and *P. aeruginosa* but was not as effective in killing *S. aureus* and this effect was not significantly different from a 5min soak in 75% EtOH. Similarly, a 5min soak in NaDCC effectively killed *S. epidermidis* but did not kill all of the *S. aureus* or *P. aeruginosa*, although the values were reduced to very low levels (0.22±0.7 and 0.11±0.33 CFUs, respectively; note that these values are not seen in Figure 2 for the sake of compactness in the graph). These levels were significantly lower than that seen after wiping. Both NaDCC and 75% EtOH were effective in killing all the three bacteria following a 10min soak in these solutions.

Figure 2 adequately and dramatically shows that the use of eye drops as a disinfectant is largely ineffective. This is particularly highlighted by the results using *S. aureus*. Even after 10min in the solution a significant bacterial load remained on the lenses, and this was not significantly different from that seen after the 5min soak. However, it should be noted that in the case of *S. aureus*, the variability in the number of CFUs was particularly high (SD±20 500).

Although chloramphenicol eye drops was not as efficacious as NaDCC or EtOH, it did appear to have significant differences between bacterial strains. There were significantly more *S. aureus* ($P\leqslant0.01$) CFUs even after a 10min soak compared to the counts obtained from *S. epidermidis* and *P. aeruginosa*; eye drops seem to have a similar influence on *S. epidermidis* and *P. aeruginosa* as CFU counts were not significantly different. *S. aureus* appeared to be significantly ($P\leqslant0.05$) more resistant to 5min in 75% EtOH compared to *S. epidermidis* and *P. aeruginosa*, while *P. aeruginosa* had the least resistance to EtOH even when wiping was used as the treatment ($P\leqslant0.05$). *P. aeruginosa* also seemed to be significantly ($P\leqslant0.05$) more sensitive to NaDCC compared with *S. epidermidis* and *S. aureus*.

Influence of Different Disinfection Methods on the Clarity of Contact Lenses The lenses were subjectively assessed and NaDCC and chloramphenicol eye drops had no effect on the clarity of the lenses (all scored 5 points). In contrast, 75% EtOH had a slight effect on the resolution of the lenses (mean scores ranged from 4.1 to 4.6). Interestingly, lenses exposed to *S. epidermidis* had the lowest scores (mean 4.1) and suggests that this bacteria may adhere more strongly to the lenses than *S. aureus* or *P. aeruginosa*.

DISCUSSION

In many ophthalmic examinations there is often direct contact with the patients' cornea[16]. Therefore, maintaining a sterile environment becomes tremendously important. Our results, consistent with previous studies[1-4,17], confirmed that the general methods currently used to disinfect contact lenses could be a risk factor resulting in cross infection in developing countries such as China. Disinfection of the tonometer head, goniolens, prism, and contact lenses has not been well addressed[1]. Bacteria infection in ocular surface in 138 pediatric subjects in Hongkong had been reported[18]. Another study of 10y retrospective study revealed that contact lens wear was the most commonly encountered risk factor for the occurrence of microbial keratitis in the pediatric age group[19]. It has been established that Hepatitis B virus can be detected in about 25% of tonometer heads[4]. This suggests that similar risks may arise from HIV, which has been found in the tears[5], aqueous humor[6], and the subretinal fluid[7]. Similar issues were raised in China during the SARS epidemic[9]. The recommendations of the Ophthalmological Society were mainly aimed at the "SARS" virus, whereas our study is primarily aimed at bacterial sources of infection. In 1985, the United States Center for Disease Control and Prevention (CDC) recommended that the contact lens should be immersed in a solution that was composed of 10% sodium hypochlorite (household bleach) and 3% hydrogen peroxide for five minutes after use[20], while the USA Contact Lens Association of Ophthalmologists recommended wiping with an alcohol-impregnated swab[21]. However, our findings show that wiping with alcohol, or indeed the other two tested disinfectants, is not the most effective measure against the bacteria tested in our experiments. Furthermore, the use of alcohol leads to some degradation of the lens quality. It is of interest to note that in a recent study by Hiller and Kumar[4], they recommended that the tonometer head should be immersed in a solution of 75% EtOH for a minimum of five minutes for disinfection. Our study is largely in agreement but shows that this is not entirely effective on one of the three bacteria (*S. aureus*), which is still present and viable on the lenses. Therefore we recommend that 75% EtOH to be used for a minimum of 10min for effective sterilization. NaDCC has the same ability to disinfect all three kinds of bacteria as EtOH, albeit slightly less effective than 75% EtOH if used as a swab wipe. Similarly, the robustness of the disinfection effect is related to the duration of soaking procedure, so we recommend a minimum of 10min as well.

Chloramphenicol is a bacteriostatic agent and is reported to be effectiveagainst *S. aureus*, *S. pyogenes* and *E. coli* but ineffective against *P. aeruginosa*, acinetobacter and enterobacter[22]. In contrast, our study showed that chloramphenicol eye drops were less effective than *S. aureus* compared to *P. aeruginosa*. Indeed, the results of disinfecting the lenses with chloramphenicol eye drops was highly variable and the least

efficacious of the three methods. We suggest that chloramphenicol eye drops should only be used as a last resort in the absence of EtOH or NaDCC.

The contact lenses used for ophthalmic examination are made from premium aircraft-grade acrylic sheets with ultra high resolution and are coated with an antireflective film to reduce the surface reflection and increase the light transmittance[23]. They should be kept away from organic solvents, high temperature conditions, chemical products and rigid materials so as not to damage the lens film and affect their resolution and appearance. Compared with high-efficiency disinfectants such as chlorine, alcohol is a medium-efficiency disinfectant but susceptible to organic compounds. In our experiment, the use of alcohol resulted in a slight decrease in the clarity of the lens. Therefore, in order to maximize disinfection but at the same time maintain lens transparency, we recommend the use of NaDCC. NaDCC not only effectively killed the bacteria after 10min but also had no effect on the clarity of the lens.

As a summary of this study, NaDCC immersion for 10min or longer appears to be a simple and effective way to disinfect contact lenses during ophthalmic examination. It is also important that the contact lenses are thoroughly rinsed after immersion to reduce the possibility of damage to the eye caused by disinfectant residue.

The trade-off with NaDCC application is its relatively long duration of disinfection time. In an understaffed ophthalmology clinic, which is the case in most developing countries, clinicians will much prefer a disinfection method with short duration. And shorten the disinfection duration may also increase the turnaround times of the lenses, so it will be more financially efficient. However, ophthalmic staffs should be alerted to the cross infection risks if the lens is not fully disinfected. At the same time, new methods that may be as or more effective but take less time should be investigated, for example ultraviolet light, microwaves or supersonic vibration with the proviso that any new method must maintain the clarity of contact lenses.

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REFERENCES

1 Chalam KV, Murthy RK. Reusable sutureless silicone ring for housing contact lens during vitreo-retinal surgery. *Retina* 2008;28(10):1550-1551

2 Gyawali R, Nestha Mohamed F, Bist J, Kandel H, Marasini S, Khadka J. Compliance and hygiene behaviour among soft contact lens wearers in the Maldives. *Clin Exp Optom* 2014;97(1):43-47

3 Nercelles MP, Peirano NL, Herrera OR, Rivero BP, Márquez PL. A nosocomial outbreak of epidemic keratoconjunctivitis. *Rev Chilena Infectol* 2010;27(6):534-538

4 Hillier RJ, Kumar N. Tonometer disinfection practice in the United Kingdom: a national survey. *Eye (Lond)* 2008;22(8):1029-1033

5 Ajayi BO, Kio FE, Otajevwo FD. Adhesive capabilities of Staphylococcus aureus and Pseudomonas aeruginosa isolated from tears of HIV/AIDS patients to soft contact lenses. *Glob J Health Sci* 2012;4(1):140-148

6 Vichitvejpaisal P, Reeponmahar S, Tantisiriwat W. Atypical manifestation of progressive outer retinal necrosis in AIDS patient with CD4+ T-cell counts more than 100 cells/microL on highly active antiretroviral therapy. *J Med Assoc Thai* 2009;92(3):S52-56

7 Ang BC, Teoh SC. Case report of cytomegalovirus retinitis in an HIV-positive patient with a CD4-count nadir of 254 cells per μL. *Eye (Lond)* 2012;26(8):1153-1154

8 Kashiwagi K, Gohdo T, Sato S, Iijima H, Tsukahara S. Detection of HIV-RNA in aqueous humor and subretinal fluid in an HIV carrier with rhegmatogenous retinal detachment. *Jpn J Ophthalmol* 2000;44(6):687-689

9 Society C. Recommendations on the disinfection work of some ophthalmologic examination devices. *Chin J Ophthalmol* 2003;39(8):1

10 De W, Huanying Z, Hui L, Corina M, Xue G, Leng L, Hanri Z, Ling F, Yanling M, Huiqiong Z, Huan Z, Jing K, Caiyun L, Yoshida H, Changwen K. Phylogenetic and molecular characterization of coxsackievirus A24 variant isolates from a 2010 acute hemorrhagic conjunctivitis outbreak in Guangdong, China. *Virol J* 2012;9:41

11Cavanagh HD, Robertson DM, Petroll WM, Jester JV. Castroviejo Lecture 2009: 40 years in search of the perfect contact lens. *Cornea* 2010;29(10):1075-1085

12 Zhao J. Ophthalmology. *People's Medical Publishing House*, 2009;306

13 Smith AF, Orsborn G. Estimating the annual economic burden of illness caused by contact lens-associated corneal infiltrative events in the United States. *Eye Contact Lens* 2012;38(3):164-170

14 Yang JW, Choi JW, Lee SG, Kim DS. Antibacterial properties of artificial eyes containing nano-sized particle silver. *Orbit* 2011;30(2):77-81

15 Yamada M, Yoshida J, Hatou S, Yoshida T, Minagawa Y. Mutations in the quinolone resistance determining region in Staphylococcus epidermidis recovered from conjunctiva and their association with susceptibility to various fluoroquinolones. *Br J Ophthalmol* 2008;92(6):848-851

16 Calkavur S, Olukman O, Ozturk AT, Kilic FK, Gulfidan G, Devrim I, Malatyali R, Oruc Y, Atlihan F. Epidemic adenoviral keratoconjunctivitis possibly related to ophthalmological procedures in a neonatal intensive care unit: lessons from an outbreak. *Ophthalmic Epidemiol* 2012;19(6):371-379

17 Choate W, Fontana F, Potter J, Schachet J, Shaw R, Soulsby M, White E. Evaluation of the PuriLens contact lens care system: an automatic care system incorporating UV disinfection and hydrodynamic shear cleaning. *CLAO J* 2000;26(3):134-140

18 Wong VW, Lai TY, Chi SC, Lam DS. Pediatric ocular surface infections: a 5-year review of demographics, clinical features, risk factors, microbiological results, and treatment. *Cornea* 2011;30(9):995-1002

19 Young AL, Leung KS, Tsim N, Hui M, Jhanji V. Risk factors, microbiological profile, and treatment outcomes of pediatric microbial keratitis in a tertiary care hospital in Hong Kong. *Am J Ophthalmol* 2013;156(5):1040-1044. e2

20 Centers for Disease Control (CDC). Recommendations for preventing possible transmission of human T-lymphotropic virus type III/lymphadenopathy-associated virus from tears. *MMWR Morb Mortal Wkly Rep* 1985;34(34):533-534

21 Updated recommendations for ophthalmic practice in relation to the human immunodeficiency virus. *J Ophthalmic Nurs Technol* 1989;8(1):15-20

22 Nam HM, Lim SK, Kim JM, Joo YS, Jang KC, Jung SC. *In vitro* activities of antimicrobials against six important species of gram-negative bacteria isolated from raw milk samples in Korea. *Foodborne Pathog Dis* 2010;7(2):221-224

23 Leem JW, Song YM, Yu JS. Six-fold hexagonal symmetric nanostructures with various periodic shapes on GaAs substrates for efficient antireflection and hydrophobic properties. *Nanotechnology* 2011;22(48):485304