Evaluation of Microbial Contamination on Contact Lenses among University Students

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A b s t r a c t
Microbial keratitis is affecting approximately 4 to 5 per 10,000 contact lens wearers worldwide and the severity of the disease depends on the type of microbial species contaminating the contact lens. As the number of contact lens wearer increases globally, including Malaysia, in the past ten years, there is a need to identify the type of microbial species that contaminates contact lenses among Malaysians, especially among college students. Therefore, this study was conducted to evaluate microbial contamination on contact lenses among university students and the habits of the contact lens wearers within the university facility. A total of 67 pairs used contact lens samples were collected. CFU/ mL was calculated based on colonies grown on nutrient agar to represent the microbial population density. Gram staining was performed for all pure cultures with different morphologies. Two major groups of contaminants with different morphologies were subjected to identification using biochemical tests. Our results suggested that 41.79 % of the samples collected were contaminated with microbes and the contamination status was significantly different between genders and duration of contact lens wearing per usage ($p < 0.05$). Besides, monthly disposable contact lenses had the highest contamination rate with a mean of 2.41 x 10^3 CFU/ mL when compared to daily and quarter-yearly (3 months) contact lenses. Gram staining showed that 88.47 % of microbial contamination was Gram negative, mainly represented by *Vibrio* spp. and *Aeromonas* spp.. Our study unexpectedly found that contact lenses among university students were contaminated with microbes that might be found in the tap water used to wash their hands.

Introduction

Recent statistics showed that around 140 million people wear contact lens and the number of contact lens wearers is increasing globally (Stapleton *et al*., 2007; Morgan *et al*., 2014). According to *Gesellschaft für Konsumforschung* (GfK) investigations, Malaysia showed the most rapid expansion in contact lens market with 15% growth rate in the first quarter of 2013 among Asian countries (GfK, 2013). An annual review of contact lens wear reported that around 70% of contact lens wearers were female among Malaysians (Morgan *et al*., 2008) and more recent studies carried out in West Malaysia revealed a higher percentage of female contact lens wearers ranging from 74% to 88% (Tajunisah *et al*., 2008; Bhandari & Rou, 2012). Tajunisah *et al*. (2008) also reported that nearly half of the subjects wearing contact lens was for cosmetic purposes and only 14.10% was for refractive correction.

The increasing number of contact lens wearers in Malaysia should be monitored seriously as knowledge and practices regarding contact lens care are still in moderate level among Malaysians

(Tajunisah *et al*., 2008; Bhandari & Rou, 2012). Previous studies showed that poor personal habits or attitude in contact lens care among wearers was one the major factors contributing to microbial keratitis (Rahim *et al*., 2008; Stapleton *et al*., 2012, Thakur & Gaikwad, 2014). Microbial keratitis is corneal infection mainly caused by bacteria agents. It affects nearly 4 to 5 per 10,000 contact lens wearers globally (Poggio *et al*., 1989; Cheng *et al*., 1999; Lam *et al*., 2002). *Pseudomonas* spp., *Staphylococcus* spp., and *Serratia* spp. have been identified as the most common bacteria contaminating contact lens and are associated with incidences of bacteria keratitis (Velasco & Bermudez, 1996, Das *et al*., 2007; Stapleton *et al*., 2012; Thakur & Gaikwad, 2014).

A study by Lam *et al*. (2011) revealed that the highest number of contact lens wearers was found between the age groups of 18 to 26 years old. Most of the individuals within this age range are university students. However, contaminants found in this group of contact lens wearers are not known. Therefore, the current study was conducted to evaluate microbial contamination on contact lenses among university students and their habit in wearing the lenses, in order to gain a better understanding on the most common microbial agents and the influence of personal habits on contamination of contact lenses. All of the subjects recruited in this study stayed in hostels within the facility of the university where some subjects shared with seven other tenants.

Methodology

Subjects and Samples Collection

Sixteen volunteers (14 females and 2 males) from different faculties in the university staying on campus were recruited for this study, regardless of age, gender, and ethnicity. Data including contact lens wearing frequency, wearing duration per usage, brand and type of contact lens, location of wearing or removing contact lens, hand washing before wearing contact lens, replacement of contact lens after expiring date and type of maintenance solution were obtained. A total of 67 used contact lens samples were collected over a period of six months. A new pair of contact lens soaked in 2 mL of sterile phosphate buffer saline (PBS), pH = 7.2, was used as the negative control.

Colony Forming Unit (CFU) and Gram Staining

All contact lens samples were vortexed briefly and 100 µL of the supernatant was plated on nutrient agar (NA) with three replicates each. For samples with high number of microbial growth, a 10-fold series dilution was performed for up to 10^{-3} until the colonies were within the countable range. All samples were incubated at 37 °C for 24 hours. Microbial population density for each sample was calculated using colony forming units (CFU/ mL). Single colonies were isolated and sub-cultured on NA to obtain pure cultures. Morphologies of the pure cultures were recorded and Gram staining was performed as described previously (Narasimhulu *et al*., 2010).

Biochemical Test

Two main groups of microbes with different morphologies (Group 1: Gram negative and rod shaped with colony morphology of round, smooth surface and white yellowish color; Group 2: Gram negative and rod shaped with colony morphology of round, smooth surface and white color) were tested biochemically using BiOLOG GN2 MicroPlate™ (BiOLOG, Hayward, CA) according to the manufacturer's instructions. The plate was visualized using BiOLOG MicroStation™ (BiOLOG, Hayward, CA) and compared to the GN Database (BiOLOG, Hayward, CA) for microbial identification.

Statistical Analysis

Statistical Package for Social Science (SPSS) V17.0 (SPSS Inc, Chicago, Illinois, USA) was used to compare the differences between selected parameters to contamination status of used contact lens using Chi-square test. The comparison is considered statistically significant if the *p*-value is less than 0.05.

Result & Discussion

Out of the 67 contact lenses collected from all subjects, 41.79% were contaminated. The negative control was free from any microbial contamination. In the current study, we found that female contact lens wearers had higher CFU/ mL. There was a significant difference between genders ($p = 0.030$) and contact lens wearing duration per usage ($p = 0.013$) (Table 1), suggesting that gender and contact lens wearing duration per usage might be two important factors contributing to microbial contamination on contact lenses. However, more samples are required to validate this finding as the sample size in this preliminary study was relatively small. Previous study in West of Scotland showed no significant difference between male and female in microbial contamination on contact lens (Devonshire *et al*., 1993) but other studies reported that males were more prone to microbial keratitis when compared to females, probably due to negligence of hygiene in males (Poggio *et al*., 1989; Dart *et al*., 1991).

Besides the two mentioned factors, ethnicity might be a candidate risk factor for microbial keratitis among Malaysians. Malaysia is a multi-ethnic country with the majority being the Malays, Chinese and Indians. Lili Asma *et al*. (2011) reported that Malaysian Chinese and Malaysian Indians had a statistically significant lower risk to contact lens-related microbial keratitis when compared to the Malays with an OR (95% CI) of 0.13 (0.05 - 0.36) and 0.30 (0.09 - 0.99), respectively. However, this aspect was not investigated in the current study.

Table 1: Contamination status with selected parameters of used contact lenses.

Selected parameters	No contamination (*N*=39)	With contamination (*N*=28)	Chi-square test (*p*-value)
Gender			0.030*
Male	7	0	
Female	32	28	
Wearing frequency			0.057
Everyday	18	15	
<4 days/week	11	1	
>4 days/week	3	5	
4-5 days/month	7	7	
Wearing duration per usage			0.013*
2-4 hours	6	0	
4-12 hours	30	20	
>12 hours	3	8	
Brand of contact lens			0.434
Bausch & Lomb	14	15	
FreshKon®	7	3	
FreshLook®	4	1	
ACUVUE®	8	7	
Others	6	2	
Type of contact lens (Mean CFU/ mL)			0.240
Daily – 1 day wearing (3)	3	1	
Daily – extended 1 day (48)	3	2	
Daily – extended 2 days (53)	1	2	
Daily – extended 3 days (80)	0	2	
Monthly (2410)	23	19	
Quarter-yearly (24)	9	2	
Location of wearing contact lens			0.573
Own bed room	20	14	
Shared bed room	10	6	
Bathroom	8	5	
Kitchen	1	3	
Hand washing before wearing			0.140
No washing	0	2	
<5 minutes without soap	12	5	
<5 minutes with soap	27	21	
Replacement after expiring date			0.111
Yes	27	14	
No	12	14	
Types of maintainence solution			0.168
Multipurpose	10	13	
Manufacturer's recommendation	8	3	
Rinsing solution	9	8	
Saline solution	12	4	

* Significant different ($p < 0.05$).

Monthly disposable contact lenses had the highest contamination rate (with a mean of 2.41 x 10^3 CFU/ mL) compared to daily and quarter-yearly (3 months) contact lenses in the current study. Surprisingly, the mean CFU/ mL for quarter-yearly contact lenses was 100-fold lower compared to monthly contact lenses. One possible explanation is that subjects using quarter-yearly contact lenses in

the current study had at least 2 years experience in wearing contact lenses and therefore had better hygiene in contact lens care. We also noticed that CFU/ mL for daily disposable contact lenses increased after extended usage (Table 1). Previous epidemiologic studies documented that extended usage of daily disposable contact lenses was related to about 0.2% of microbial keratitis and had five times higher risk to microbial keratitis when compared to contact lenses that were disposed after daily usage (Poggio *et al*., 1989; Schein *et al*., 1989). It is therefore not recommended to extend the maximum usage duration of contact lens to minimize the risk of microbial keratitis. In the current study, none of the subjects had a history of microbial keratitis.

Gram staining in the current study revealed that 88.47 % of the microbial contaminant was Gram negative and rod shaped, including 44.23 % with colony morphology of round, smooth surface and white yellowish color (Group 1) as well as 32.70% with colony morphology of round, smooth surface and white color (Group 2) (Table 2). Most of the previous studies showed that Gram negative bacteria such as *Pseudomonas* spp. and *Serratia* spp. were frequently isolated from contact lenses (Das *et al*., 2007; Goh *et al*., 2010; Stapleton *et al*., 2012) but some studies showed that Gram positive bacteria like *Staphylococcus* spp. and *Streptococcus* spp. were more commonly contaminated on contact lens (Velasco & Bermudez, 1996; Szczotka-Flynn *et al*., 2010). The difference in the type of bacteria isolated could be due to geographical differences, which may influence the type of bacteria species isolated from contact lenses (Shah *et al*., 2011; Willcox, 2012).

Table 2: Percentage of colony morphology and Gram staining for microbial contamination on contact lenses.

Colony morphology	Gram staining	Percentage (%)
Round, smooth surface and white yellowish color	Negative, rod	44.23
Round, smooth surface and white color	Negative, rod	32.70
Rough surface and white color	Positive, bacillus	7.69
Round, smooth surface and yellowish color	Negative, rod	7.69
Round, smooth surface and pink color	Negative, rod	3.85
Rough surface and white color	Positive, rod	1.92
Round, smooth surface and white yellowish color	Positive, cocci	1.92

By using biochemical tests, we unexpectedly found that the most abundant contaminant, Group 1, was predominantly *Vibrio* spp., whereas Group 2 contaminant was mainly *Aeromonas* spp.. These two species are usually isolated from water and aquatic environments (such as in tap water, seawater and groundwater) (Igbinosa & Okoh, 2010; Janda & Abbott, 2010). About 97.01 % of the subjects in the current study washed their hands with tap water before wearing contact lens and this might be the key source of contamination for *Vibrio* spp. and *Aeromonas* spp., especially if the subjects did not dry their hands before touching the contact lenses. Although contamination of *Vibrio* spp. and *Aeromonas* spp. on contact lenses are rare, Pinna *et al*. (2004) reported a microbial keratitis case associated with contact lens contaminated with *Aeromonas* spp. and the irregular rinsing of the contact lenses with tap

water. Furthermore, rinsing contact lens cases with tap water instead of disinfectant solution may also increase the risk of bacteria contamination (Rahim *et al*., 2008).

Conclusion

The current study showed that gender and contact lens wearing duration per usage had a significant influence on contamination status on contact lenses. However, larger sample size is necessary for further validation. We also found that most of the microbial contaminants on the collected contact lenses were Gram negative bacteria and the majority of contaminants were *Vibrio* spp. and *Aeromonas* spp., which are commonly found in tap water. We recommend campaigns or talks regarding contact lens care to be conducted in Malaysia to educate contact lens wearers to enhance awareness and practices in contact lens care and to minimize the risk of microbial keratitis.

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References

Bhandari, M. & Rou, H. P. 2012. Habits of contact lens wearers towards lens cars in Malaysia. *Medical Journal of Malaysia*, 67: 274-277.

Cheng, K. H., Leung, S. L., Hoekman, H. W., Beekhuis, W. H., Mulder, P. G., Geerards, A. J. & Kijlstra, A. 1999. Incidence of contact-lens-associated microbial keratitis and its related morbidity. *The Lancet*, 354: 181-185.

Dart, J. K., Stapleton, F. & Minassian, D. 1991. Contact lenses and other risk factors in microbial keratitis. *The Lancet*, 338: 650-653.

Das, S., Sheorey, H., Taylor, H. R. & Vajpayee, R. B. 2007. Association between cultures of contact lens and corneal scraping in contact lens-related microbial keratitis. *Archives Ophthalmology*, 125: 1182-1185.

Devonshire, P., Munro, F. A., Abernethy, C. & Clark, B. J. 1993. Microbial contamination of contact lens cases in west of Scotland. *British Journal of Ophthalmology*, 77: 41-45.

GfK SE. 2013. Contact lens sales in six key Asian markets generated over USD660 million in first three quarters of 2013. GfK Press Releases. Nuremberg, Germany.

Goh, P. P., Shamala, R., Chandamalar, S. & Tai, X. Y. 2010. Contact lens-related corneal ulcer: a two-year review. *Medical Journal of Malaysia*, 65: 120-123.

Igbinosa, E. O. & Okoh, A. I. 2010. *Vibrio fluvialis*: an unusual enteric pathogen of increasing public health concern. *International Journal of Environmental Research and Public Health*, 7: 3628-3643.

Janda, J. M. & Abbott, S. L. 2010. The genus *Aeromonas*: taxonomy, pathogenicity, and infection. *Clinical Microbiology Reviews*, 23: 35-73.

Lam, D. S., Houang, E., Fan, D. S., Lyon, D., Seal, D. & Wong, E. 2002. Incidence and risk factors for microbial keratitis in Hong Kong: comparison with Europe and North America. *Eye (London)*, 16: 608-618.

Lam, D. Y., Kinoshita, B. T., Jansen, M. E., Mitchell, G. L., Chalmers, R. L., McMahon, T. T., Richdale, K., Sorbara, L. & Wagner, H. 2011. Contact lens assessment in youth: methods and baseline findings. *Optometry & Vision Science*, 88: 708-715.

Lili Asma, I., Lekhraj, R., Hejar, A. R., Nazri, O, Habshah, M. & Azrin Esmady, A. 2011. Risk factors for contact lens related microbial keratitis. *Medicine & Health*, 6: 270.

Morgan, P. B., Woods, C. A., Knajian, R., Jones, D., Efron, N., Tan, K. O., Pesinova, A., Grein, H. J., Marx, S., Santodomingo, J., Runberg, S. E., Tranoudis, I. G., Chandrinos, A., Itoi, M., Bendoriene, J., van der Worp, E., Helland, M., Phillips, G., Gonzalez-Meijome, J. M., Belousov, V. & Mack, C. J. 2008. International contact lens prescribing in 2007. *Contact Lens Spectrum*, January: 36.

Morgan, P. B., Woods, C. A., Tranoudis, I. G., Helland, M., Efron, N., Teufl, I. M., Grupcheva, C. N., Jones, D., Tan, K. O., Pesinova, A., Pult, H., Ravn, O., Gierow, P., Santodomingo, J., Malet, F., Plakitsi, A., Vegh, M., Erdinest, N., Chande, P. K., Ragnarsdottir, J. B., Itoi, M., Chu, B. S., Bendoriene, J., van der Worp, E., Awasthi, S., Lam, W., Leszczynska, W., Gonzalez-Meijome, J. M., Belousov, V., Silih, M. S., Hsiao, J. & Nichols, J. J. 2014. International contact lens prescribing in 2013. *Contact Lens Spectrum*, 29: 30-35.

Narasimhulu, K., Rao, P. S. & Vinod, A. V. 2010. Isolation and identification of bacteria strains and study of their resistance to heavy metals and antibiotics. *Journal of Microbial & Biochemical Technology*, 2: 074-076.

Pinna, A., Sechi, L. A., Zenetti, S., Usai, D. & Carta, F. 2004. *Aeromonas caviae* keratitis associated with contact lens wear. *Ophthalmology*, 111: 348-351.

Poggio, E. C., Glynn, R. J., Schein, O. D., Seddon, J. M., Shannon, M. J., Scardino, V. A. & Kenyon, K. R. 1989. The incidence of ulcerative keratitis among users of daily-wear and extended-wear soft contact lenses. *The New England Journal of Medicine*, 321: 779-783.

Rahim, N., Bano, H. & Naqvi, B. S. 2008. Bacterial contamination among soft contact lens wearer. *Pakistan Journal of Ophthalmology*, 24: 93-96.

Schein, O. D., Glynn, R. J., Poggio, E. C., Seddon, J. M. & Kenyon, K. R. 1989. The relative risk of ulcerative keratitis among users of daily-wear and extended wear soft contact lenses: a case-control study. *The New England Journal of Medicine*, 321: 773-778.

Shah, A., Sachdev, A., Coggon, D. & Hossain, P. 2011. Geographic variations in microbial keratitis: an analysis of the peer-reviews literature. *British Journal of Ophthalmology*, 95: 762-767.

Stapleton, F., Edwards, K., Keay, L., Naduvilath, T., Dart, J. K. G., Brain, G. & Holden, B. 2012. Risk factors for moderate and severe microbial keratitis in daily wear contact lens users. *Ophthalmology*, 119: 1516-1521.

Stapleton, F., Keay, L., Edwards, K., Naduvilath, T., Brain, G. & Jacobs, R. 2007. Studies of contact lens-related microbial keratitis in Australia and New Zealand: new learning. *Eye Contact Lens*, 33: 354-357.

Szczotka-Flynn, L. B., Pearlman, E. & Ghannoum, M. 2010. Microbial contamination of contact lenses, lens care solutions, and their accessories: a literature review. *Eye Contact Lens*, 36: 116-129.

Tajunisah, I., Reddy, S. C. & Phuah, S. J. 2008. Knowledge and practice of contact lens wear and care among medical students of University of Malaya. *Medical Journal of Malaysia*, 63: 207-210.

Thakur, D. V. & Gaikwad, U. N. 2014. Microbial contamination of soft contact lenses and accessories in asymptomatic contact lens users. *Indian Journal of Medical Research*, 140: 307-309.

Velasco, J. & Bermudez, J. 1996. Comparative study of the microbial flora on contact lenses, in lens cases, and in maintenance liquids. *International Contact Lens Clinic*, 23: 55-58.

Willcox, M. D. P. 2012. Management and treatment of contact lens-related *Pseudomonas* keratitis. *Clinical Ophthalmology*, 6: 919-924.