Journal of Academia Vol. 9, Issue 1 (2021) 106 – 116

Relationship between Routine Handling Practices with Potential Pathogenic Bacteria Isolated from Contact Lenses Among Students in UiTM Negeri Sembilan

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Abstract

Pseudomonas aeruginosa and *Staphylococcus aureus* are types of bacteria known to cause bacterial keratitis. *Pseudomonas aeruginosa* causes bacterial keratitis by adhering to the surface of the contact lenses, when the *P. aeruginosa* are in contact with the eye, resulting in infectious keratitis. As for *Staphylococcus aureus*, when there is a predisposing factor such as wearing expired or extended use of contact lenses (contact lenses that can be used continually for up to one week even while sleeping) weaken the individual defences and leads to the development of bacterial keratitis. Both bacteria are capable to infect eye cornea and lead to bacterial keratitis through contact lenses wearer. The findings of this study provide information on the importance of routine practices in handling contact lenses to help reduce the incidence of bacterial keratitis caused by wear contact lenses in an individual. The side effect of wearing contact lenses such as redness of the eye and keratitis due to the infection by pathogenic bacteria which comes from the behavior and low hygiene level management of individual had led the study to create awareness to contact lenses wearer. In this study, 25 soft and hard contact lenses with purposed for colored or toric contact lenses were obtained among UiTM Negeri Sembilan students. The users required to answer the questionnaire form regarding the type, behavior, and routine practices of their contact lenses. Pathogenic bacteria were isolated using the cotton swab technique and cultured on nutrient broth. The streak technique was used to cultured bacteria from broth to nutrient agar, blood agar, and MacConkey agar. Later, the identification of bacteria was carried out using biochemical tests and microscopic observation. From the laboratory results, 84% of the tested contact lenses contained pathogenic bacteria on their surface. These findings concluded that the presences of pathogenic microorganisms on the contact lenses used closely related to the behavior in handling and hygenic practices level by the contact lenses users.

Keywords: Pathogen, contact lenses, bacterial keratitis.

Article History:- Received: 7 November 2020; Accepted: 7 March 2021; Published: 30 April 2021

Introduction

An estimated number of individuals that wear contact lenses around the world is 125 million (Janumala *et. al*, 2012). In Malaysia, the estimation of individual wearing contact lenses is between 6 to 7% of the population and the range age for the contact lens users is between 20 to 30 years old group (Ismail *et al.*, 2016). Contact lenses are often worn for medical, lifestyle or cosmetic purposes (Fatin *et al.*, 2019). Until now, there are only two types of contact lenses in the market which are soft contact lenses and gas-permeable lenses which also known as hard lenses (Phani and Gaurav, 2018). The difference between soft and hard lenses is soft lenses more flexible but less durable while hard lenses are rigid and more durable (Heiting, 2016). The medical contact lenses can be classified into many types such as myopia (near-sightedness), hyperopia (far-sightedness), astigmatism (distorted vision) and presbyopia (need for bifocal). Meanwhile, cosmetic lens is classified based on the different color and size.

Despite serving multiple purposes to aid vision, prolong use and improper after-use care of the contact lenses may cause and lead to many issues, such as frequent eye infection, severe allergies, dry eye, bacterial keratitis, blurry vision, eye feels scratchy and can lead to blindness if not treated properly. The statistics of bacterial keratitis infection in Malaysia from 2006 to 2013 stated that 47.2% of the cases were caused by contact lenses usage (Nazri et al, 2017). The study of bacterial keratitis in Sarawak shows that the most common pathogen isolated was *Pseudomonas aeruginosa* (22 out of 38 eyes), followed by *Staphylococcus aureus* (1 out of 38 eyes) (Bou *et al.,* 2019). *Pseudomonas aeruginosa* is commonly found in soil, water, and plant, and often is related to secondary bacterial infection to cornea damage due to its complex genetic makeup such as mechanism for adaptation, survival and resistance thus provide the ability (Karpecki and Shechtman, 2012). According to Wu *et al,* (2015), the *P. aeruginosa* will initially adhere to the surface of the contact lenses or its carrying case, before infecting the cornea resulting in infectious keratitis. *Staphylococcus aureus* on the other hand, is a non-motile Gram-positive bacteria and member of Bacilli and normal flora of skin. However, the bacterium can also be pathogenic toward human, where one research mentioned that 30% of humans were colonized and infected by *Staphylococcus aureus* (Kobayashi *et al.*, 2015). This pathogen act through predisposing factor for keratitis such as wearing expired or prolong use of contact lenses will weaken the individual mucous membrane defence (O'Callaghan, 2018).

Many people are unaware of proper handling and hygienic way of handling contact lenses. Another risk factors that can lead to bacterial keratitis are by wearing the contact lenses longer than the prescribed hours, sleeping while wearing contact lenses, extended wear contact (contact lenses that can be used continually for up to one week even while sleeping), and poor hygiene of lens and the storage case. Many pathogens are resistance to antibiotics and able to develop a new resistance after been introduced to any antimicrobial agents (Moradali *et al.,* 2017). Therefore, this study aims to identify the presence of *P. aeruginosa* and *S. aureus* on contact lenses surface and to determine the routine practice in handling the contact lenses among tested students in Universiti Teknologi Mara (UiTM) Negeri Sembilan. The findings from this study provide information which help in creating awareness to reduce the incidence of bacterial keratitis caused by the contact lenses among the users.

Methods

Questionnaire set

The questionnaire set were conducted in google form. The questionnaire set were developed according to the risk factor of wearing contact lenses referring to Fatin *et al.,* (2019). The respondents were questioned on the hygienic behavior in handling the contact lenses. Google form that consisted of questions regarding the personal background, type and behavior of contact lenses and hygiene level practices toward contact lenses including types of storage case and cleaner solution. Scope of answer range is according to Fatin *et al.,* (2019) and the question need to be answer within one week before handover the contact lenses. The questionnaire form was answered randomly by 25 respondents who wear contact lenses within UiTM Negeri Sembilan (UiTM Kuala Pilah, UiTM Rembau and UiTM Seremban) and all the respondents are between age of 18 to 23 years old. These criteria were referred to a finding of previous study conducted by Ismail *et al*., (2016) stated that the common range of individual wore contact lenses was 20 to 30 years old. The individual that answer the questionnaire set will hand over their contact lenses for lab experiment.

Sample collection

25 contact lenses that obtained from the respondents were to be determined and evaluated whether respondents aware of the relationship between hygiene and behavior management related to the existence of pathogenic bacteria on the surface of contact lenses. The contact lenses were collected directly from respondents together with the storage case and label according to the name of the owner and from which UiTM. The storage cases separated independently in a sterile plastic bags and immediately kept inside the refrigerator with the temperature of -4°C to provide sterile condition toward the contact lenses.

Isolation of potential pathogenic from samples

The isolation technique was referred to Bou *et al.,* (2019) by using sterile cotton swab. Forceps was used to hold the contact lenses in order to prevent contamination during isolation. Next, sterile cotton swab was used to streak the surface of contact lenses and soak into the nutrient broth and incubated at 37 °C for 16-24 hours. The cultured broth was streaked onto the surface of nutrient agar by using an inoculating loop. The tested media was incubated at 37 °C for 16-24 hours until the pure culture obtained. The characteristics of colony grow were recorded.

Selective agar identification method

In this method, 2 selective agars were used namely; MacConkey agar and Blood agar that encourage the growth of bacteria that able to lyse blood such as *S. aureus* and *P. aeruginosa*.

The pure colony from nutrient agar were streak onto MacConkey agar and blood agar, respectively. The tested media were incubated at 37°C for 24 hours until the matured colony obtained. The positive result of MacConkey agar showed that colony change colored from yellow to pink or red and proved that the organism is Gram-negative bacteria as MacConkey agar inhibited the growth of Gram-positive bacteria. Negative results showed that the organism is a fastidious organism where the bacteria need particular or special nutrients to growth and blood was not the nutrient needed by the organism.

Microscopy Observation

The colonies on the nutrient agar was isolated and observe under a compound microscope through two techniques which was Gram staining and Hanging drop technique. Then, Gram staining technique was used to identify the shape and whether the organism was Gram-positive or Gram-negative bacteria. The procedures were referred to Takenaka *et al.,* (2012). Four reagents used in the technique which was crystal violet, Lugol's iodine, acetone alcohol solution, and safranin. After that, observation of dry-stained smear under microscopic observation with 100x magnification and will showed blue or purple-colored bacteria for Gram positive while for Gram-negative bacteria, the red or pink-colored bacteria. Other than that, hanging drop technique was referred to Nazri *et al.,* (2017) used to observe the motility patterns of the microorganism. Firstly, a drop of broth culture was placed onto a clean coverslip that was encircled with Vaseline. Then, the coverslip with wet mount smear was then inverted over the well of a concave slide. Lastly, the culture was hung from the coverslip and observe under the microscope to observe the motility of the sample.

Biochemical tests

There are 5 series of biochemical tests done to identify the pathogenic bacteria that presence in the contact lenses; Oxidase test, Catalase test, Sucrose test, Citrate test and TSI test. The method used for all biochemical tests were referred to Hindmarsh and Geer (2017) with minor modification.

Oxidase test was designed to determine the ability of the organism to produce the cytochrome oxidase enzyme. A drop of Kovac reagent was placed onto a filter paper. Then, a pure culture was picked using a blunt wooden tooth pick and placed onto the filtered paper. The inoculated area of paper was observed for a color change within 10-30 seconds. Positive results showed the conversion and oxidized of colorless Kovac's reagent to purple color (bacteria able to produce cytochrome C oxidase enzyme) and the negative result showed no color change of Kovac's reagent. Positive control bacteria used was *Pseudomonas aeruginosa* and negative control used was *Staphylococcus aureus* obtained from UiTM Kuala Pilah laboratory sample.

Catalase test was designed to identify the ability of an organism to produce catalase enzyme. Hydrogen peroxide solution was drop onto a clean slide. The pure colony was picked from Nutrient agar using the sterile wire loop and placed onto the reagent drop. The reaction appeared immediately and will be recorded. Positive result showed bubble formation and no bubble formation for negative result. Positive control becteria used was *Staphylococcus aureus* while negative control used was *Streptococcus pyogenes* obtained from UiTM Kuala Pilah laboratory sample.

Sucrose test was designed to identify whether the organism can ferment sucrose. Phenol red was used as an indicator to detect the lowering pH of the medium. A few drops of sample broth were placed into the sucrose test bottle. The tested media was incubated at 37°C for 24 hours. Positive result showed the change color of medium from red to yellow. The medium for negative result showed no change in color. Positive control bacteria that used was *Pseudomonas aeruginosa* and negative control used was *Staphylococcus aureus* obtained from UiTM Kuala Pilah laboratory sample.

Citrate test was designed to differentiate between Gram-negative bacilli or Gram-negative rod bacteria (Aryal, 2019). The sample from pure colony on Nutrient agar will be streaked onto the surface of citrate media. Next, the citrate media was incubated at 37°C for 24 hours. The positive result was the change color of citrate agar from green to blue. Negative results showed no change color of citrate agar. Positive control bacteria that used was *Pseudomonas aeruginosa* and negative control used was *Escherichia coli* obtained from UiTM Kuala Pilah laboratory sample.

Triple Iron Sugar (TSI) test was designed to identify the ability of sample or organism to produced gas, the formation of Hydrogen Sulfide (H_2S) gas and ferment sucrose, lactose, and glucose. The sample from pure colony on Nutrient agar were stab into the media and streak onto the surface of the media using straight wire. Precaution that the media was not break off during this streaking procedure. The media was incubated at 37°C for 24 hours. Positive result for glucose fermented showed the change color of agar slant from red to yellow while negative result showed no change color of agar slant. Positive results for sucrose and lactose fermented showed the change color of butt agar from red to yellow while negative results showed no change color. The positive result of the H_2S test showed the formation of black precipitate on top slant of agar while negative result no formation of black precipitate on top of agar. Positive result of gas formation showed bubble or cracks formed within the agar while negative result for gas production is no bubble or cracks within the agar. All the result of biochemical test was compared and identified the type of the bacteria. Control was TSI agar incubated without organism stab into the media.

Data analysis

Statistical analysis that was used in this study was the Chi Square test. It was used to compare and relate the factors from the questionnaire set with the finding obtained from the laboratory test of the individual sample. The result of the laboratory test for each test was marked by using numbered and compared with the numbered sample. This statistical analysis is performed to presence of negative pathogenic bacteria on surface of contact lenses according to risk factor. Odd ratio(OR) is a measure strength of association with an exposure and an outcome as OR more than 1 means greater likelihood of having association with the exposure and outcome, while OR equal to 1 means there is no association between exposure and outcome and OR less than 1 means there is low likelihood of association between exposure and outcome.

Result and Discussion

Among all 25 students that involved in this study, (64%) of the user were female and (46%) were male. Most of the students in this study using soft lenses that been used for a month. Majority of the respondents wear soft contact lenses (84%) as soft contact lenses is more comfortable and easier to wear compare to hard contact lenses. In the meantime, 52% of the respondents wear the contact lenses daily, while 68% of the respondents wear contact lenses more than 8 hours in a day and 92 % of the respondents wear the monthly disposable contact lenses which can increase the chance of bacterial keratitis as the contact lenses may expose or have high growth of bacteria on the lenses surface. A total of (84%) of the respondents wear contact lenses for medical purpose while (16%) of the respondents wear contact lenses for cosmetic purpose. 54% of the respondents were aware the misuse or overextend use of contact lenses may lead to bacterial keratitis and other 46% of respondent does not aware of it.

Table 1. Type and behaviour of contact lenses users

Variables	Frequency	Percentage (%)
Gender		
Male	9	36
Female	16	64
Type of Contact Lenses		
Hard Lenses	4	16
Soft Lenses	21	84
Often Wear of Contact Lenses		
Daily		
Rarely	13	52
	12	48
Duration wear Contact Lenses		
< 8 hours		
≥ 8 hours	8	32
	17	68
Contact Lenses Due Date		
Daily Disposable	2	8
Monthly Disposable	23	92
Contact lenses purpose		
Medical	21	84
Cosmetic	4	16
Aware misuse of contact lenses may lead to bacterial keratitis		
Aware	13	52
Unaware	12	48

Also, among all 25 students that involved in this study, individual hygiene level management of study also have been answered where most of the respondents did not often wash their hands before applying and handling contact lenses (68%). In the meantime, only (32%) of correspondents clean their contact lenses before and after use the contact lenses by using cleaning solutions which increase the probability of being infected by bacterial keratitis if not always cleans the contact lenses before and after use. (72%) of the correspondent change the cleaning solution in the storage case sometimes or occasionally which is also can be considered risk factor toward accumulation of bacteria inside the storage case and adhere to the contact lenses. Lastly, 48% of the correspondent strictly follow the instruction of cleaning the contact lenses which is mostly using the rubbing technique to clean their contact. This method can be considered a risk factor when the owner of contact lenses does not wash hands and use rubbing technique to clean their contact lenses, the bacteria on the hand surface may adhere to the contact lenses during rub the contact lenses.

Table 2. Individual hygiene level management

Variables	Frequency	Percentage (%)
Wash Hand before Handling Contact lenses		
Always	8	32
Sometime	17	68
Cleaning Contact Lenses Before and After Use Contact Lenses		
Always	8	32
Sometime	17	68
Frequency Changing Cleaning Solution in Storage Case		
Always	7	28
Sometime	18	72
Cleaning Contact Lenses Strictly Follow the Instruction		
Yes	12	48
No	13	52

From the screening tests on the presence of microorganism showed that 21 samples were inhabit with bacteria. There were 2 patterns of bacterial colony on the Nutrient agar. The first group of bacteria showed greenish coloration due to development of Pyoverdine pigment, the colonies also showed large, opaque, flat colonies with irregular margin. This pathogen has only one type of appearance when grown on blood agar, the colonies that can be found when growth on blood agar is golden-yellow colonies with beta hemolysis (β-hemolysis) (Snehalatha *et al.*, 2016; Tankeshwar, 2013). The enzyme that was produced by the bacteria to cause the complete lysis of the blood cell is Streptolysin which is an exotoxin (Aryal, 2019). Around 6/25 (24%) samples showed the similar appearances as mentioned above that leads to highly suspected as *S. aureus*. Next, the bacterial colonies were also inhibited with non-fermenter colonies on MacConkey agar. Through microscopic identification approach, the bacteria appeared blue-purple coccus in arrangement of chain under Gram staining smear and do not show motility behavior under hanging drop observation. The first group of bacteria were suspected to be *S. aureus.* While the second group showed colonies with a golden yellow endopigment coloration due to development of carotenoid pigment, the colonies also showed circular colonies with convex elevation on the nutrient agar. About 15/ 25 (60%) samples showed metallic sheen and mucoid colony on Blood agar as shown in Figure 1. The samples were heavy growth on the MacConkey plate with no pink or red color appearance. Under microscopic observation, the bacteria appeared pink-reddish rod in single arrangement under Gram staining smear and the bacteria have single with no sheath polar flagellum that helped the bacteria for motility behavior under hanging drop observation. These bacteria were suspected to be *P. aeruginosa*.

Table 3. Result of colonies growth on agar plate

Bacterial Group	Nutrient Agar	Blood Agar	MacConkey Agar	Number of Sample
First group	Greenish coloration	Golden-yellow colonies with beta hemolysis (β-hemolysis)	Negative growth	6/25 (24%)
Second group	Golden yellow endopigment coloration	Metallic sheen and mucoid colonies	Grow with no pink color appearance	15/ 25 (60%)

Figure 1. *Pseudomonas aeruginosa* colonies growth on blood agar

The samples were undergoing biochemical tests for further identification of the bacteria. As for the first group of bacteria that suspected as *S. aureus*, only catalase and oxidase testing were conducted on these bacteria to determine whether the bacteria either *S. aureus* species or other *Streptococcus* species (Tankeshwar, 2012). The result showed that the bacteria produce negative result of oxidase test as the bacteria cannot produce cytochrome c oxidase, but positive result of catalase test showed that the bacteria can produce catalase enzyme. All of the findings confirmed the first group as *S. aureus*. As for the second group of bacteria that were suspected as *P. aeruginosa* colonies, the other biochemical tests such as oxidase, catalase, citrate, sucrose and TSI agar test were conducted. As for oxidase test and catalase test, the result showed that the suspected *P. aeruginosa* bacteria were positive result proved that the bacteria can produce catalase enzyme and cytochrome c oxidase enzyme. As for sucrose test, the result showed that there is change or color of sucrose phenol red solution from red to orange and yellow color and proved that the bacteria can ferment sucrose in the media. For citrate test, the result showed that 80% of the suspected *P. aeruginosa* colonies was positive result and proved that the bacterial able to utilize citrate as the source of energy and growth on the medium (Lili *et al.,* 2016). While 20% of the suspected *P. aeruginosa* colonies showed negative result due to the error during handling or little amount of growth's organism on the slant of the agar. Lastly, for the TSI agar test toward the suspected *P. aeruginosa* colonies showed the slant and butt of the agar is red color means that the organism does not ferment glucose, sucrose, and lactose and in alkaline condition (K/K). The suspected bacteria do not produce of H_2S as there is no black color of ferrous sulfide on the agar (Cremers *et al.,* 2014). The second group of bacteria also do not produce any gas as no crack or bubbles on the TSI agar. Based on Hindmarsh and Geertsma (2017), all the findings for this study meet characteristics showed by *P. aeruginosa,* as expected.

Table 4. Biochemical test first group of bacteria

Bacterial Group	**Catalase test**	**Oxidase test**	**Bacteria**
First group	Positive result	Negative result	*Staphylococcus aureus*

Table 5. Biochemical test second group of bacteria

Bacterial Group	**Catalase test**	**Oxidase test**	**Sucrose**	**Citrate**	**TSI agar**				**Bacteria**
					Butt	**Slant**	**Gas**	**H_2S**	
Second group	Positive result	Positive result	Red to yellow	12/15 green to blue	K	K	No gas	No black color	*Pseudomonas aeruginosa*

The isolates were labelled according to their type of contact lenses, name and cross checked with the info in questionnaire set to identify their behavior and hygiene level management. After all identification tests were done, the finding showed that there were 24 % of the samples showed the presence of *S. aureus*, 60% had *P. aeruginosa* and 16 % were sterile as in the chart of Figure 2.

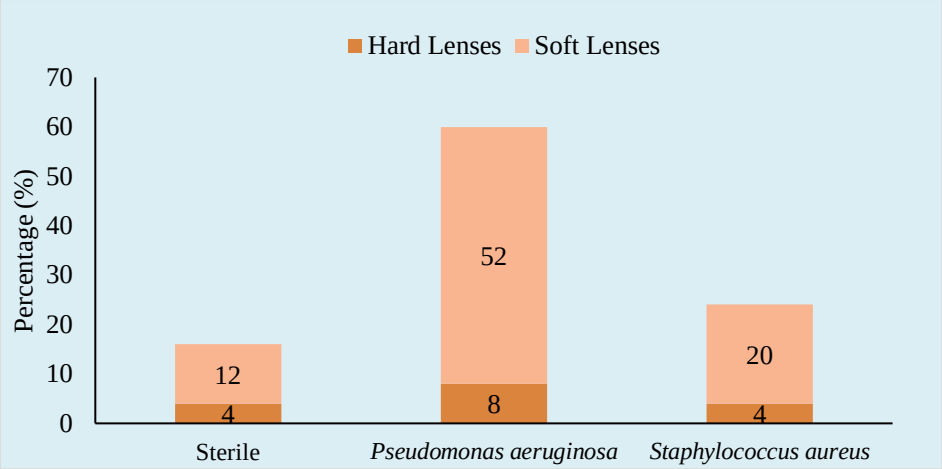

Figure 2. The percentage of potential pathogenic bacteria found in 25 samples

Based on the statistical analysis of the data in Table 1, most of the users in this study choose the soft lenses compared to hard lenses, however the possibility being contaminated with pathogen was high with odd ratio more than 1 (OR=2.0). There is a significant relationship between the type of contact lenses against the presence of pathogenic bacteria on the surface of contact lenses. The previous research done stated that soft lenses have better delivery of oxygen to eye cornea but have a high possibility to cause irritation and bacterial contamination (Loh and Agarwal, 2010). The other three factors that showed significant relationship with the presences of pathogenic bacteria on the surface of contact lenses were the prolonged duration of wearing, not washing hands before applying the lenses and rarely cleaning the contact lenses before and after used (OR =1.5). Based on the previous research mentioned that the contact lenses that being wore more than 8 hours can lead to the formation of pathogenic bacteria between eye cornea and contact lenses surface which permits the infection to the users eye cornea (Bedinghaus, 2019). Hand however was the common place for the normal flora such as *S. aureus* to adhere which may become an opportunistic pathogen and caused bacterial keratitis (O'Callaghan, 2018). Thus, wash hands with sanitizer or soap is a must before handling and apply contact lenses on the eye. The other practices that should be taken in consideration were washing lenses before and after wearing the lenses. By ignoring it, the lenses will be easily contaminated.Washing lenses before and after wearing the lenses is important as the solution were able to clean, rinse, disinfect and store contact lenses to reduce probability of potential pathogenic bacteria adhere toward the contact lenses (White, 2017).

The most prominent factor of behavior that affecting the cleanliness of lenses was frequency in changing the cleaning solution. The users that rarely change the cleaning solution in the storage case has a higher risk of bacterial keratitis caused by lenses with odd ratio value < 1 (OR = 0.0784, P value = 0.0470) compared to the users that frequently changing the cleaning solution in the storage case. The storage solution that never or occasionally changing has a high possibility to be contaminated with pathogenic bacteria and later the bacteria adhere to the surface of contact lenses. Thus, it is important to frequently change the solution in the storage case to kill and prevent the growth of bacteria on the storage case solution.

Table 6. The relationship between the risk factor and presence of pathogenic bacteria on contact lenses surface that lead to bacterial keratitis

Risk Factor	Positive Pathogenic Bacteria (N=21)	Negative Pathogenic Bacteria (N=4)	Odds Ratio (OR)	95% Confidence Interval (95% CI)	Overall P Value
Type of Lenses					
Soft Lenses	18	3	2.000*	0.1527 - 26.1886	0.5974
Hard Lenses	3	1			
Pattern wear Contact Lenses					
Daily	10	3	0.3030	0.0270 - 3.4072	0.3335
Rarely	11	1			
Duration wear Contact Lenses					
< 8 hours	7	1	1.500*	0.1310 - 17.1804	0.7445
≥ 8 hours	14	3			
Contact Lenses Due Date					
Daily Disposable	1	1	0.1500	0.0073 - 3.0918	0.2191
Monthly Disposable	20	3			
Wash Hand before Handling Lenses					
Always	7	1	1.500*	0.1310 - 17.1804	0.7445
Sometime	14	3			
Cleaning Contact Lenses Before and After Use					
Always	7	1	1.500*	0.1310 - 17.1804	0.7445
Sometime	14	3			
Frequency Changing Cleaning Solution in Storage Case					
Always	4	3	0.0784*	0.0064 - 0.9667	0.0470*
Sometime	17	1			
Clean Contact Lenses Follow Instruction					
Yes	10	2	0.9091	0.1071 -7.7185	0.9304
No	11	2			

Description: * shows relationship at 95% confidence level

Finally from the finding, there are three factors that do not affecting the prevalance of contaminated pathogenic bacteria on the lenses. Which is contact lenses due date, not following the instruction of washing technique and pattern of wear contact lenses does not have the significant relationship against the presence of pathogenic bacteria on the surface of contact lenses.

Conclusion

The pathogenic bacteria that presence on the surface of the contact lenses collected among students of UiTM Negeri Sembilan are *P. aeruginosa* and *S. aureus.* The potential isolates that were found similar characteristis with pathogenic bacteria were 84% out of 25 samples obtained. The risk factors determined from the findings were related with type of contact lenses, duration of wearing it, hygenic measures taken before handling the lens including washing hand, cleaning the lens before and after the

use and the frequency of changing cleaning solution in the storage case. Eventhough wearing contact lens itself brings the possibility to experience bacterial keratitis, improve the level of hygenic practise can reduce the possibility to eye infections prevalance. The future study should focus the quantitative study on the amount of pathogen and to conduct the molecular identification of the pathogenic bacteria on the surface of contact lens.

Acknowledgement

The authors are grateful to Universiti Teknologi MARA (Cawangan Negeri Sembilan) for supporting the laboratory apparatus and facilities to conduct the experimental work. Not to forget, thank you to all students of UiTM Negeri Sembilan that participate as the respondents in this research.

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