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Qualitative Analysis of Biofilm Formation and Control of E.coli, Pseudomonas and Candida species

Afreen Saleem[1]

[1]Jinnah university for women, Karachi Pakistan.

ABSTRACT:

Biofilm formation in food processing industry may lead to several chronic diseases such as typhoid, shigellosis, listeriosis, cholera etc. The monitoring and control of pathogens in processing lines are crucial in order to avoid contamination of products. Various disinfectants (iodine, methanol, chlorine, dettol) were taken to observe their effect on axenic biofilm and xenic biofilm. The following study evaluated the anti-biofilm effect of disinfectant solutions against single specie and multi species biofilm formation on stainless steel surface. Ten minuteexposure with sanitizing solutions that are iodine, methanol, chlorine and dettol had significantly reduced the cell counts. The resistance against iodine was observed in both types of biofilm and CFU count on single species biofilm was 4.48x10^8 and of the multi species biofilm was5.2x10^8. Methanol showed to be good in killing for single species biofilm while multi species biofilm was resistant to it and it CFU count on macConkey agar was 1.7x10^9. Chlorine and dettol cause total degradation in the cell counts of both types of biofilm. The present study showed that, multi species biofilms are more resistant to disinfection as compared to mono specie biofilm. It ought to be taken into consideration that chemical disinfection technique shall give a fascinating, efficient result and not inflicting any adverse result in human health. Additionally to, the chemical disinfectants like chlorine and dettol, have a progressive role to play in preventing food borne illness.

Keywords: *biofilm, disinfectant, resistance, xenic, axenic*

INTRODUCTION

A biofilm is a colonization of various microbes; that are belong to various different groups of sessile microorganisms (e.g. Bacteria and fungi) that are aggregate together which can be termed as Microcolonies. This mainly occur due to the poor hygiene of food, contact surface, utensils, and environments. These all play role in contamination of food which leads to the food borne diseases. Many of the bacteria are involve like, *Listeria monocytogenes*, *Escherichia. coli* and *Salmonella* (Boulange-Peterman, and others *et at.* 1993). Cross contamination also play important role in contaminating the food and environment. (Barnes, *et al.*, 1999, Boulange Peterman, 1996). Frank and Chmielewski, 1997) and Holah,. 1990) verified that the kind of food and source of surface along with topography play a significant role in the inability to decontaminate a surface. Rough surfaces accumulate soil and are more difficult to clean than smooth surfaces. Surface defects provide protection beside the elimination of dust and microbes (Boulange-Peterman, 1996), but they are capable of regrow and become colonize. Various form of biofilm are more resistant to disinfectants, which may assist the survival of Listeria spp and, other food borne pathogens in the food processing environment. Direct evidence that the pathogen-containing biofilms play a role in the spread of foodborne illness is lacking, as identification and characterization of biofilms has not been

included in food borne illness investigations. There is the number of various sorts of biofilms as there are microbes, and even one bacterium might make a few unique sorts of biofilms under various natural conditions. It is comprised of microbial cells and an extensive variety of self-produced extracellular polymeric substances (EPS), including polysaccharides, nucleic acids, and proteins. Biofilm arrangement is a dynamic procedure, which is composed by the connections of various microbial species. Be that as it may, the vast majority of the systems with respect to biofilm development are uncovered by a method for concentrating on mono-species biofilms. (Liang Yang1, 2011)

Biofilms constitute a one of a kind method of development that permits survival in antagonistic situations. Scientists have assessed that 60-80 percent of microbial diseases in the body are created by microscopic organisms developing as a biofilm. Biofilm microbes are a part of what is known as the Th1 bacterial pathogens, which as indicated by the Marshall Pathogenesis, all things considered reason perpetual ailments. Specifically, biofilms show expanded imperviousness to compound cleansing, antimicrobial treatment, and human safe reactions (Costerton, *et al.*, 1995) (Hall-stoodley, 1999). These sorted out groups are crucial to guarantee a natural balance as the occupants of biofilms are described by their survival under unpleasant conditions, for example, drying up or supplement starvation and their investment in the worldwide bio-geochemical cycle (Burmølle, *et al.*, 2012). Multi species biofilms are without a doubt the prevailing structure in nature and are likewise unmistakable in the human host, for instance, in the oral hole and the lungs of cystic fibrosis (CF patients. Biofilms are additionally found in man-made situations, where they might be identified with nosocomial contaminations, nourishment decay, and harm to modern pipelines (Stoodley, P., 2004). The colonization of various pathogenic types of local tissues, for example, the lung of cystic fibrosis patients, perpetual injuries, or the urinary tract every now and again incites more serious and unmanageable diseases (Wolcott, 2013). Nourishment contact surfaces are regularly treated with disinfectants and cleaning specialists that contain peroxides, chloramines or hypochlorites. Specifically, the last can be extremely forceful to stainless steels relying upon the over arching pH. It is very much reported that microscopic organisms, including foodborne pathogens, for example, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli*, can "stick" to an assortment of surfaces found in nourishment commercial enterprises (Cabeça T.K, *et al.*, 2010). a study demonstrated that a Bacillus subtilisstrain isolated from an endoscope washer-disinfector, which was especially resistant to the high groupings of oxidative disinfectants utilized day by day as a part of these tools, could secure S.aureus from the activity of per acetic corrosive inside of a multispecies biofilm (Bridier, *et al.*, 2012). So also, it was shown in a late work that resident flora from lettuce builds S. typhimurium imperviousness to UV-C light in these natural surroundings (Jahid, *et al.*, 2015). The nearby area and complex communications inside biofilms underlie both synergistic and antagonistic practices. For instance, species inside of a biofilm can seek healthful assets or on the other hand can arrange to better use supplements or withstand brutal conditions. The multi species biofilms are ubiquitous and found in both natural and clinical situations; one can expect that synergistic communication between species prevail over hostile ones, especially cooperative energies that encourage a vigorous conjunction (Periasamy and Kolenbrander, 2009). A few studies show that oral microscopic organisms depend on interspecies cooperation's when shaping multi spp biofilms, with every species assuming a particular part (Filoche, *et al.*, 2004, Sharma *et*

al., 2005) (Yamada *et al.*, 2005). Antiseptics and disinfectants are widely use in other health care settings has various other applications. Variety of vigorous chemical agents are available commercially, few of them are alcohols, phenols, iodine, and chlorine. They carry broad-spectrum antimicrobial activity; though, little is known about the mode of action of these agents in comparison to antibiotics (Gerald McDonnell, 1999). Mode of action is Similar to chlorine, the antimicrobial action of iodine is rapid, even at low meditation, mode of action is still unknown; but has higher penetration rate in to various microorganisms.(Chang, 1971) where they are capable of act on key groups of proteins (particularly free-sulfur amino acids cysteine and methionine, nucleotides, and fatty acids (Apostolov, 1980), which culminates in cell death. Alcohols are rapidly carried out bactericidal activity instead of bacteriostatic activity to kill vegetative cells of bacteria; they also have positive effects against Tuberculi bacilli, Fungai and viruses but are not able to kill spores of bacteria. On the other hand Methyl alcohol has lowest bactericidal action of the alcohols and thus seldom is used in healthcare.

Manufactured biofilm model frameworks are utilized much of the time by scientists to perform more particular and reproducible biofilm considers. For instance, in the flow chamber biofilm development framework, microscopic organisms labeled by fluorescent proteins are vaccinated into little glass chambers and observed all through biofilm improvement by utilizing CLSM (Sternberg C, 2006).

MATERIAL AND METHOD

Bacterial strains: The following strains of bacteria were used in this study: *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida spp.*

Growth Media: Muller Hinton broth, Tryptic soy broth, Tryptic soy agar, Brain heart infusion broth, macConkey agar.

Disinfectant: Iodine solution, methanol, chlorine, dettol.

Equipment: Shaking water bath, centrifuge machine, spectrophotometer, Autoclave, CO2 Incubator, Bath sonicator, cell imager, UV-hood, test tubes, petri dishes, tissue culture flask, steel coupons.

Identification of bacterial strains: Cultures of E.coli and Pseudomonas were cultivated on macConkey agar for 24 hours in an aerobic atmosphere at 37°C. Escherichia coli (lactose-positive) and Pseudomonas aeruginosa (lactose-negative) on MacConkey agar. The oxidase and catalase test for P.aeruginosa showed positive results. Further confirmation of these bacteria was done by microscopy. Cultivation of Candida on sabouraud's dextrose agar, colonies were white to cream colored, smooth, glabrous and yeast-like in appearance. Microscopic morphology shows spherical to subspherical budding yeast-like cells.

Preparation of bacterial suspension for biofilm: The bacterial strains were grown overnight (18 to 24h) at 37°C with shaking (150 revolutions per minute-rpm) in tryptic soy broth. Cells were harvested by centrifugation at 5,000 x g for 3.5 min and washed three times with phosphate-buffered saline (PBS; 0.1M, pH 7.2). Cell pellets were resuspended in PBS and adjusted by a spectrophotometer to an A660 of approximately 0.5, corresponding to ~ 108 CFU/ml.

Disinfectants: The disinfectants used in this study were chosen to represent those used in the food industry. The following disinfectants were used: iodine solution, methanol, chlorine and Dettol.

Test surface: AISI type 304 stainless steel, the surface chosen as it is used extensively throughout the food processing industry. Flat, stainless steel coupons (1 x 1cm) were used as the test surface to examine the biofilm formation in vitro. The coupons were initially

soaked overnight in acetone to remove grease. After soaking, the steel coupons were placed in a sterile tube and sonicated for 15 min in a bath sonicator. The coupons were then washed in tap water followed by three washes with distilled water, and they were autoclaved at 121°C for 15 min. The manipulations of coupons were assisted with a sterile surgical clamp for all assays.

Biofilm formation in vitro: A 60µl aliquot of the 108 CFU/ml suspension prepared as described above was placed in tissue culture flask containing 15 ml of inoculated Mueller Hinton Broth and one sterilized stainless steel coupon all the defined procedures took place in a laminar flow hood to avoid contamination for both types of biofilm. The tissue culture flask was then incubated in CO2 incubator. The culture medium was discarded every 3rd day of incubation and replaced by freshly prepared BHI+ sucrose. After 2 weeks of incubation biofilm formation was confirmed by using cell imager.

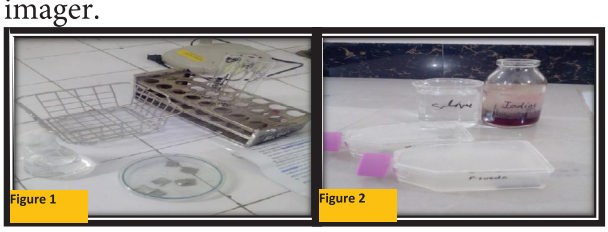


Figure 1: Preparation for sterilizing stainless steel coupons and Figure 2: Disinfection of biofilm with Iodine.

Activities of the tested disinfectants against the biofilm cells:

Suspension test for disinfectants:

Principle: A sample of bacterial culture from biofilm is suspended in the disinfectant solution.

After exposure it is verified by sub-culturing whether this inoculum is killed or not.

Suspension tests are preferred as the bacteria are uniformly exposed to the disinfectant.

Qualitative suspension test: After biofilm formation, coupons were rinsed twice with 5 ml of sterile physiological saline to remove any attached bacterial cells, and then they were soaked in 5ml of iodine solution for 10 minutes and again rinsed with sterile saline. The same procedure was performed by using methanol, chlorine and dettol.

To quantify viable cells after the process of disinfection, steel coupons were again observed through cell imager. And biofilm organisms were suspended, serially diluted 10-fold with sterilized physiological saline and cultured in macConkey agar at 37°C for 24–48h.

Quantitative suspension test: A number of surviving organisms (B) are counted and compared to the original inoculum size (A).

Microbicidal Effect (ME) = Log (A) – Log (B)

ME= 1 □killing about 90% of the initial number.

ME= 2 □ 99% killing

A general accepted requirement is:

ME>5 □ 99.9% of the germs are killed.

RESULT:

Biofilm formation in any health service or human interaction environment can be injurious to mankind. To control their formation cleaning agents should be used. In this study, we have formed two types of biofilms single specie and multi species biofilm and for its control we use various disinfectants. Biofilm formation was observed by using cell imager as it is shown in figure 7 and 8. We have used iodine, methanol, chlorine and dettol to remove biofilm from the stainless steel surface. Cultures were taken from biofilms and serially diluted in order to count cfu on macConkey agar plates. After disinfection with iodine, both biofilms have not shown major destruction. Colony forming unit, counted for single spp biofilm was 4.48x10^8 and multi spp biofilm was 5.2x10^8. Single spp biofilms were killed

Table I: The effect of disinfectants on single specie and multi species Biofilm

Disinfectant	Effect on Single spp Biofilm	CFU/ml	Effect on Multi spp Biofilm	CFU/ml
Iodine	Growth	4.48x10^8	Growth	5.2x10^8
Methanol	No growth	_	Growth	1.72x10^9
Chlorine	No growth	_	No growth	_
Dettol	No growth	_	No growth	_

Table II: The resistance or susceptibility of Single specie biofilm against disinfectants.

Single species biofilm	Iodine	Methanol	Chlorine	Dettol
	Resistant	Susceptible	Susceptible	Susceptible

Table III: The resistance or susceptibility of Multi species biofilm against disinfectants.

Multi species biofilm	Iodine	Methanol	Chlorine	Dettol
	Resistant	Resistant	Susceptible	Susceptible

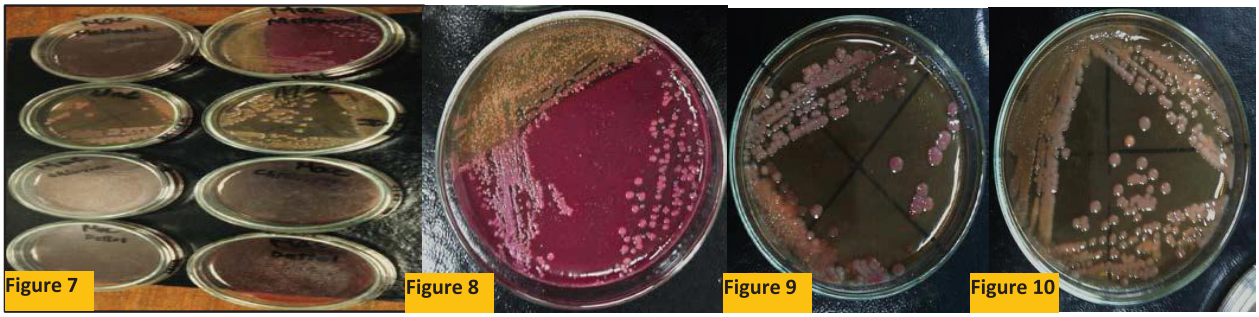


Figure 7: The growth of subculture organisms after disinfection on MacConkey agar. **Figure 8:** The Multi species biofilm after disinfection with Methanol. **Figure 9:** The Single specie biofilm after disinfection with Iodine. **Figure 10:** The Multi species biofilm after disinfection with Iodine.

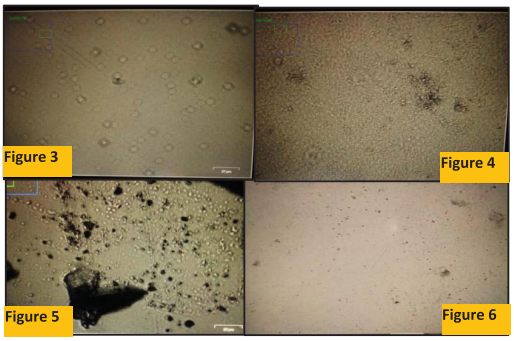
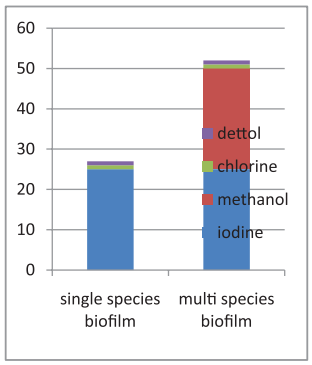


Figure 3: indicates Multi species biofilm before disinfection. **Figure 4** indicates E.coli biofilm before disinfection. **Figure 5** indicates Multi species Biofilm. After disinfection **Figure 6** indicates E.coli Biofilm After disinfection.

by all other disinfectants that were used while multi spp biofilm showed resistance against methanol as demonstrated in table 2 and 3. Chlorine and dettol cause thorough destruction of both forms of biofilms.

DISCUSSION:

Food borne illnesses are always problematic to the human all over the world. Biofilms are responsible for the different incidences. It is huge hallmark in the food industry as well; they also require various chemicals and disinfectants. Although microbial interactions between bacteria have been studied primarily in planktonic culture systems, these are more likely to occur in multi species biofilms in which genetically distinct bacteria may become attached to one another via specific molecules.



Graph 1: The biofilms resistance against disinfectants.

As described above in result section, We have performed disinfection of two types of biofilms that are mono specie biofilm and multi species biofilm the findings of our research demonstrate that mono specie biofilm exhibited a significant decrease in the survival rate of viable cells after treatment with tested disinfectants as compared to dual species biofilm. No growth was detected after 10 min of exposure to chlorine, dettol and methanol in mono specie biofilm while growth is observed after disinfection with Iodine that is (4.48x10^8 cfu/ml). According to the previous investigation almost similar results were observed (Simões, Pereira, & Vieira, 2005). Moreover, Disinfection of mono specie biofilm when compared with disinfection of multi species biofilm it showed resistance against both the iodine and methanol that was 5.2x10^8 cfu/ml and 1.72x10^9 cfu/ml. Chlorine and dettol proved to be good in killing of multi species biofilm too. Corresponding results were observed in previous investigations (Andrade, et al., year).

It may be more difficult to compare results from different studies because the conditions for attachment and biofilm development vary greatly and use of variety of disinfectants must have significant differences.

Chemical agents like chlorine and dettol were seen to be finest to eliminate biofilm cells formed on stainless steel surfaces, while iodine seems to be the worst. While earlier studies showed dissimilar findings that iodine at sub-bactericidal concentrations demonstrates molecular and enzymatic effects that are highly associated with biofilm formation (Avshalom Tam., 2006).

Bacterial interactions may be accomplished through extracellular compounds whose sole role is to influence gene expression, metabolic co-operativity and competition, physical contact, and the production of the antimicrobial exo-product. One or all of these may be occurring simultaneously and begin to influence a biofilm during the initial stages of formation, bacterial attachment, and surface colonization and continue to influence the structure and physiology of the biofilm as it develops. Further studies should be performed to further investigate how and why bacteria growing in complex surface-attached communities can protect themselves from the action of antimicrobial agents.

CONCLUSION:

Food also provides a source to all pathogenic organisms where they can form biofilm. Therefore, it will contaminate the food. Fresh food is essential requirement for all humans health, so it is needed that to develop such techniques by which food can be processed commercially. There must be predictable control strategies are used and must be develop, that are capable of food safety at cheaper rate. These procedures includes such disinfection methods that are advantageous, cheaper and has no health hazards n human and environment.

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Detection of D-Xylose Activity of Yeast Species Isolated from Local Yoghurt and Sugar Cane Juice in Karachi

Leila Mohammadi[1]

1 Jinnah university for women, Karachi, Pakistan.

ABSTRACT

Biofuels are vigor transporter that can hoard energy strained from biomass. There are various forms of biomass which are derived from food, wood and fiber; that can be obtain by processing slug of industrial, agricultural, forestry material. About twenty samples of yoghurt, and sugar cane juice were purchased indiscriminately from various places of Karachi. About 10 strains of yeasts were isolated by using Yeast extract peptone agar. Each strain was examining on the basis of cultural, morphological and biochemical characteristics. The total numbers of yeast strains were 10 from yoghurt while 5 from sugarcane extract. These isolates have ability for the production of diverse extra cellular enzymes by fermenting different sources of carbon; in resultant alcohol is produced.

Keywords: *Biofuels, biomass, slug.*

INTRODUCTION

Sugarcane (Saccharum officinarum) is economically is amongst the world's economically chief crops all around the world. Pakistan is at 4th position for the production of Sugar cane. Worldwide it is cultured on 20. 42 million ha with an estimated total manufacture of 1333 million metric tons (Agribusiness Handbook, 2009). Biomass goes through a complex four-part process to generate ethanol, which is used as a fuel in place of or in addition to conventional petroleum products (Preez, 1994). Raw materials, both grain and cellulosic biomass, are first pretreated in order to begin breaking down the material and generating more surface area for the second step, hydrolysis (McMillan, 1994). Hydrolysis is accomplished either by the use of enzymes or chemicals. In this step the complex carbohydrate chains in the biomass are broken down to simple sugars. Finally, these sugars are fermented by microorganisms, yeast, fungi, or bacteria, which produce ethanol in a dilute form. In order to concentrate the ethanol, distillation techniques are used. If pure ethanol is required, the product is subjected to further separation techniques (Visser, 1990). Microorganisms that quickly hydrolyze sugar xylose to ethanol with great output; it is necessary to develop such techniques for the production of ethanol that are cost effective and able to produce at large scale. Certain fungi and bacteria are capable to ferment xylose to ethanol has been recognized for many years (Simon, 2005). A variety of wild-type fungal and bacterial genera can carry out direct fermentation of xylose. Fungal genera, frequently show increase production but undergo from low rates, include Fusarium, Monilia, Mucor, Neurospora, Paecilomyces, Polyporus, and Rhizopus. Furthermore bacterial species includes both from mesophilic and thermophilic genera (Dien, 2003). As the need of ethanol is increasing; it is required that to isolate such strains that produce increase amount of ethanol by using such techniques that require low budget. So it can be available in market at cheaper rate (Sures 1999; Suresh 1999). One of the process is yeast cell immobilization that is prudent as compare

to other commercially available systems, it can provide faster rate of fermentation. The cost is low due to in situ removal of cells (Chaudhary, 1996). It also protects cells from the lethal effects of acidic pH, osmotic pressure, inhibitors, temperature etc which play essential role in high ethanol production at cheaper rate (Sree NK, 2000).

METHODOLOGY

Collection of samples: Samples of yoghurt, and sugarcane juices, were collected randomly from local markets of different areas of Karachi in sterile bottles and kept at 4°C.

Isolation of yeast strains: The samples were serially diluted, plated on a selective medium, yeast extract peptone dextrose agar and were incubated at 28°C for 48 hours. The colonies appeared were further purified.

Morphological characterization: The strains were stained by lacto phenol-cotton blue, carbol fuchsin and seen under phase contrast microscope.

Determination of alcohol producing ability: Table 1: Morphological, Biochemical and Physiological characteristics of the yeast strains isolated from yoghurt Assimilation

RESULT & DISCUSSION

20 samples of yoghurt, and sugar cane juice were monitored for bioethanol production from where 15 yeast strains were isolated and purified (Table I and II). Maximum yeast strains were isolated from yoghurt samples, followed by sugarcane juice. Most of the isolated colonies exhibited smooth surfaces with circular margins. The color of the colonies showed a wide variation of creamy white and pinkish. The cells were found to be of various shapes such as round; oval, spherical and ellipsoidal. (Table I and II). The physiological researches of each yeasts strain were carried out by using over 1 test for assimilation of carbon and their catalase activity. The utilization of xylose was tested. The biochemical analysis of the strains isolated from yoghurt and sugar cane samples showed that all the strains could grow in presence of sugars and urea and ferment them (Table I and II respectively). The surface, margin and color of the colonies isolated from the various samples differed from each other. However, smooth, circular and creamy white colonies were found to be more prevalent. All the strains isolated were found to assimilate the xylose and produce detectable amount of xylanase respectively. The strains also exhibited catalase ability of various degrees. The result of this study indicated that most of the indigenous yeasts, isolated from yoghurt and juice samples showed good fermentation attributes, which might enhance ethanol yield that would contribute for the cost effective role in the production of bioalcohol and enzymes of industrial importance.

Yeast strain	Surface	Margin	Color	Glucose Sugar / Alcohol	Fructose Sugar / Alcohol	Sucrose Sugar / Alcohol	Maltose Sugar / Alcohol	Urea Sugar / Alcohol	D-xylose	Catalase activity
D1	Smooth	Irregular	Creamy White	++/+	+++/+	++/ +	++/ +	-/+	+	+
D2	Rough	Circular	Creamy White	++/+	+++/+	+++/+	+++/+	-/+	++	+
D3	Smooth	Circular	Creamy White	+++/+	+++/+	+++/+	+++/+	-/+	++	+
D4	Rough	Irregular	Creamy White	++/+	+++/+	++/+	++/+	-/+	++	++

Yeast strain	Surface	Margin	Color	Glucose Sugar / Alcohol	Fructose Sugar / Alcohol	Sucrose Sugar / Alcohol	Maltose Sugar / Alcohol	Urea Sugar / Alcohol	D-xylose	Catalase activity
D5	Rough	Irregular	Creamy White	++/+	+++/+	+/+	+/+	-/+	++	+
D6	Rough	Circular	Whitish black	+/+	+++/+	++/ +	++/+	-/+	++	+
D7	Rough	Irregular	Whitish black	++/+	+++/+	+/+	++/+	-/+	++	++
D8	Rough	Irregular	Creamy White	++/+	+/+	+++/+	+++/+	-/+	++	+
D9	Rough	Irregular	Creamy White	++/+	++/+	+++/+	+++/+	-/+	+	+
D10	Rough	Irregular	Blackish	++/+	+++/+	+++/+	+/+	-/+	+	++

Table 1: Morphological, Biochemical and Physiological characteristics of the yeast strains isolated from yoghurt Assimilation

Figure 1

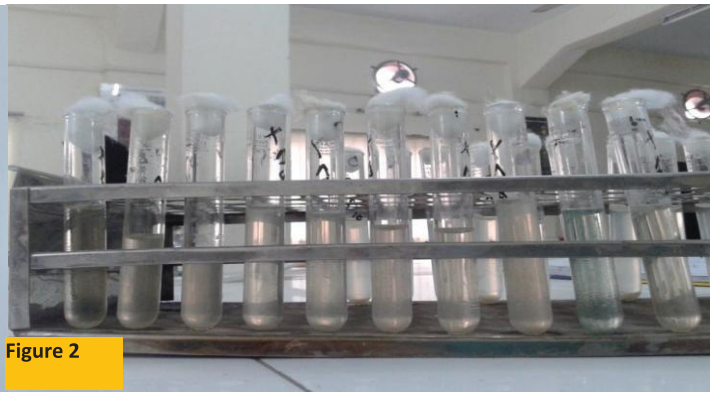

Figure 2

Figure 1: Yeast isolated from yoghurt and sugar cane samples.

Figure 2: Production of alcohol from D- xylose.

Yeast Strain	Surface	Margin	Color	Gluc. Sugar / Alcohol	Fruc. Sugar / Alcohol	Suc. Sugar / Alcohol	Malt. Sugar / Alcohol	Urea Sugar / Alcohol	D- xylose	Catalaes activity
A1	Rough	Irregular	Creamy white	+/+	++/+	+++/+	+/+	++/+	++	+
A2	Rough	Irregular	Creamy white	+++/+	++/+	+++/+	++/+	++/+	++	+++
A3	Smooth	Circular	Creamy white	+++/+	+++/+	+++/+	++/+	+++/+	+++	+
A4	Rough	Circular	Creamy white	+++/+	+++/+	+++/+	++/+	++/+	++	++
A5	Smooth	Circular	Creamy white	+++/+	++/+	+++/+	++/+	+++/+	+	++

Table 2: Morphological, Biochemical and Physiological characteristics of the yeast strains isolated from Sugar cane Assimilation

CONCLUSION

Our aim was to produce bioethanol from yoghurt and sugar cane. There are various techniques available industrially. Though, the leading apprehension of the process is its cost. For this we must use some cost efficient bioethanol production process and in our study we used one of the most cost efficient products for bioethanol production and we hope that some efficient research on these procedures will make them used in daily industrial procedures for bioethanol production.

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Microbiological Analysis of Ophthalmic Solutions

Sana Siraj Khan, Amna Shafiq & Syeda Ghufrana Nadeem

Department Of Microbiology, Jinnah University For Women, Karachi.

ABSTRACT

Microbial corneal contamination is the most genuine confusion of contact lens wear. A defiled contact lens arrangement and embellishments can go about as a supply for microorganisms that could conceivably agree contact lens wear and prompt sight debilitating unfavorable occasions. The rate of lens case arrangements ruining is normally more than half. The point of our exploration study to detach microorganisms from contact lens stockpiling arrangements and to describe and recognized by front line morphology, common characteristics and biochemical test. We take distinctive specimens of lens consideration stockpiling arrangement from asymptomatic patients. Culture tests on blood agar and macconkey agar for gram positive and negative microscopic organisms, SDA for contagious separation and PAS (Periodic acid–Schiff) for Acanthamoeba segregation. Furthermore, we discovered 100% lens care stockpiling arrangements debased from microorganisms. In which our outcome demonstrates that half specimens were defiled with gram positive microorganisms while half examples were tainted with gram negative microscopic organisms, and 67% Fungus were available. Acanthamoeba were not disengaged in any tried specimen, might be on the grounds that it once in a while separated invitro from tests. Subsequently it is reduced that it is critical to keep up abnormal state of contact lens cleanliness and that you tail all the cleaning systems. Genuine consideration ought to be taken by contact lens clients, to keep up abnormal state of cleanliness, appropriate changing of lens consideration arrangement after some time.

Keywords: *contactlenses, Acanthamoeba, hygiene*

INTRODUCTION

Visual diseases rate increments considerably nowadays and stick out amongst the most critical component is the expanded utilization of contact lenses. Contact lenses (CLs) are presently worn by a huge number of individuals worldwide and by around 1.65 million individuals in the UK (Roberts A, Kaye AE, *et al*, 2005). The wearing of CLs causes changes in the cornea as far as structure, turnover, and tear generation, oxygen and carbon dioxide levels. These adjustments in themselves can create issues and may likewise compound prior conditions, and results in various sorts of visual infections. A late study has found that they represent 9.1% of the referrals into the eye setback unit (Melia B, Islam T, *et al*, 2008). There is expanding proof that bacterial biofilm assume a part in an assortment of visual diseases. Bacterial development is described as a biofilm when microorganisms join to a surface and/or to each of other (Michael E. Zegans, *et al*, 2004). Microbial keratitis is a possibly blinding infection that is uncommon in typical eyes unless connected with contact lens (CL) (J.K.G. Dart, FCOphth, *et al*, 1991). Visual parasitosis in human is more common in land territories where ecological components and poor clean conditions support the parasitism in the middle of man and creatures. The main danger elements for Acanthamoeba keratitis are contact-lens wear and corneal. Despite the fact that >80% of the instances of Acanthamoeba keratitis happen in contact

lens. Clinical appearance of Acanthamoeba keratitis is outspread neuritis and serious agony that is not similar with the degree of tissue harm injury (Amal R. Nimir, Ahmed Saliem, and Ibrahim Abdel Aziz Ibrahim, *et al*, 2012). Parasitic keratitis was characterized as a corneal epithelial imperfection with basic stromal penetrate, Any atypical, infiltrative injury with badly characterized or sporadic edges, satellite or stretching injuries, and particularly in the nearness of a ring invade would raise the suspicion of contagious keratitis (Wei-Boon, Khor Tin Aung, *et al*, 2006). The range of causative agents in all microbial keratitis changes by atmosphere and inclining element. As a rule, Gram-positive microbes are all the more every now and again recuperated in calm atmosphere regions(Bourcier T, Thomas F, *et al*, 2003) whereas Gram-negative bacteria and their growth is crucial in tropical or sub-tropical atmospheres (Fong CF, Tseng CH, *et al*, 2004). Growths represent 5–40% of culture demonstrated contaminations. Pseudomonas aeruginosa is the most normally recuperated causative living being in contact lens-related sickness, trailed by Gram-positive microbes, parasites and Acanthamoeba (Galentine PG, Cohen EJ, *et al*, 1984). The lens, stockpiling case, and visual environment may offer a suitable survival corner for this ecological life form. P. aeruginosa can stick to and colonize lens materials amid wear and make due in contact lens stockpiling cases (Szczotka-Flynn LB, Pearlman E, Ghannoum M, 2010). Numerous include basic and intense to-regard microorganisms known as Staphylococcus aureus. In any case, the most extreme might be Pseudomonas aeruginosa, a quickly developing bacterial disease that can prompt an opening in your cornea. Infections likewise known not genuine eye diseases they might be transmitted from contact lenses of ailing patient, Herpes and adenoviral diseases can happen amid CL wear, Although HIV has been disconnected from visual tissues, tears and delicate CLs utilized by patients with AIDS, contact lenses might be a critical vehicle for the exchange of microorganisms from tainted contact lens answers for the cornea.

MATERIAL AND METHODS

Sample Collection: To isolate microorganisms from contact lens storage cases, we take different samples of lens care storage cases solution from asymptomatic patients. Sample was collected by swabbing on different media as according to the requirement.

Bacterial Isolation: Using a standard 5, ul bacterial loop, the lens case solution was cultured onto 5% Columbia blood agar and MacConkey agar plates and incubated in air at 30°C for 3 days. After incubation all lactose fermenting and non-lactose fermenting Gram negative bacilli were identified to the genus level using a series of manual biochemical tests." These tests included; Oxidase, motility, oxidation/fermentation, citrate, methyl red, indole, Voges-Proskauer, and growth on Macconkey agar. Catalase was assayed qualitatively using hydrogen.

Peroxide as outlined in the Manual of Clinical Microbiology.'1 Gram positive bacteria were identified by their characteristic colonial morphology and Gram stain appearance. No attempt was made to further speciate bacteria.

Fungal Isolation: A 0.5 ml aliquot of the lens case solution was cultured onto an SAC slope (Sabouraud dextrose agar + chloramphenicol 0.1% + gentamicin 0'4%) and incubated in air at 27°C. Cultures were incubated for 14 days before being discarded as negative. Positive cultures were identified by microscopic and macroscopic morphology.

Amoebal Isolation: A 1 0 ml aliquot of the contact lens case solution cultured on a Pages amoeba saline (PAS) agar plate. PAS which had previously been spread with a lawn of heat killed (65°C/30 minutes) Escherichia coli.1I The

Table 1: Morphological, Cultural & Biochemical Characteristics of the isolated gram positive bacteria

Number Of Sample	Colonial Characteristics On Ba	Cultural Characteristics	Gram R/C	Catalase	Coagulase	Growth On Msa	Identified Organism
SAMPLE#1	Gray white colonies with beta hemolysis	Cocci in chains	+ve	-ve		-	Streptococcus
SAMPLE#2	Large round yellow colonies with beta hemolysis / and gray colonies	1.Cocci in clusters/ 2.Cocci in chains	+ve	1.+ve 2. -ve	1. +ve 2. -ve	1.-ve 2.+ve	1.Staphylococcus 2.Streptococcus
SAMPLE#3	Large colonies with beta hemolysis	Scattered rods	+ve	+ve	-	-	Bacillus

Table 2: Morphological, Cultural & Biochemical Characteristics of the isolated gram negative bacteria

Number of samples	Colonial morphology	Cultural characteristic	Gram R/C	TSI	citrate	urease	oxidase	Identified organism
Sample#4	Small round non-lactose fermenting colonies	Scattered rods	-ve	K/A	+ve	-ve	+ve	Pseudomonas aeruginosa
Sample#5	Small round non-lactose fermenting colonies	Scattered rods	-ve	K/A	+ve	-ve	+ve	Pseudomonas aeruginosa
Sample#6	Large gummy lactose fermenting colonies	Rods	-ve	A/A	+ve	+ve	-ve	Klebsiella

Number of sample	Macroscopic characteristics	Microscopic characteristics	Fungus identified
Sample#4	Black colonies	Dark brown round conidia	Aspergillus niger
Sample#3	1.Black colonies 2.velvety white colony	1.dark brown conidial heads 2. sporangiospores, branched hyphae	Aspergillus niger/mucor
Sample#5	Black colonies	dark brown conidial heads	Aspergillus niger
Sample#6	Powdery / smoky green	1.Conidial heads & short conidiophores 2. septate hyphae & long conidiophores	Aspergillus fumigates/ Aspergillus flavus

plates were incubated in a humidified chamber at 30°C for up to 7 days, and examined every 48 hours. Cultured amoebae were identified as either Acanthamoeba or other free living amoebae by morphology of cyst, flagellate, and trophozoites stages.

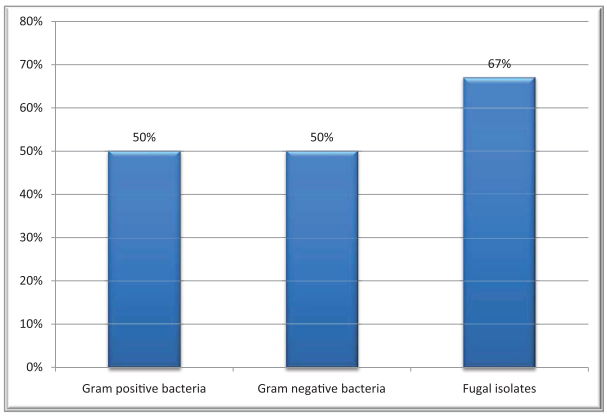

Fig. 1: Prevalence rates of microorganisms in ophthalmic solutions

RESULTS

Microbiological analysis of contact lenses showed increased level of microbial contamination. All the samples were contaminated with microbes except amoeba. Out of 6 samples, 3 were contaminated with gram positive bacteria (33.3% streptococcus, 16% staphylococcus, 16% Bacillus) while other three samples were contaminated with gram negative bacteria (33.3% *Pseudomonas aeruginosa*, 16% Klebsiella). Fungus was isolation four out of six samples (50% *Aspergillus niger*, 16% *Aspergillus flavus*, 16% *Aspergillus fumigatus*, 16% sporangium). Acanthamoeba were not isolated in any tested sample, may be because it rarely isolated invitro from samples.

DISCUSSION

Corneal disease is the most well-known vision debilitating complexity of contact lens wear. Living beings disengaged from contact lens related corneal ulcers have frequently been appeared to be indistinguishable to those confined from the contact lens case, reaching lens arrangement a conceivable load wellspring of pathogenic organisms. Regardless of the obvious adherence to prescribed cleaning and sanitizing administrations, a noteworthy level of microbial pollution of contact lens arrangements was found in this study. In our investigation a huge level of microbial defilement of contact lens stockpiling arrangement was found in this study. Large portions of the contaminants recognized were potential pathogens and in that capacity ought to have been averted by the disinfectant treatment utilized. In our examination study distinctive bacterial strains (100%) and parasitic strains (80%) detached and protozoa were not separated in our study. This study highlights that there is a requirement for development in contact lens stockpiling arrangement cleanliness. Current contact lens sterilization strategies don't have all the earmarks of being giving an alluring level of Microbial assurance. It was accounted for in the past study that most regular microbial contaminant secluded in descending prevalence were microorganisms (78%)>fungi (24%)>protozoa (20%). About all contact lens cases had blended microbial populaces. The most widely recognized bacterial contaminants detached were non-fermentative. Gram negatives took after by coliforms, other Gram negative, and Gram positive living beings (Trevor B Gray *et al.*, 1995). In our examination concentrate exceedingly pathogenic living beings were disengaged, Pseudomonas can be spread from the hands in the lens care arrangement that gets violated and is not appropriately cleaned causes ophthalmic diseases. Streptococcus was likewise confined; Streptococcal diseases are a vital reason for corneal ulcers, endophthalmitis, conjunctivitis, and dacryocystitis. Staphylococcus is additionally exceptionally pathogenic microscopic organisms causes ophthalmic diseases. There are various approaches to get Staphylococcus and Streptoccus diseases once you have a tear or damage in your cornea. Disease spread when eye comes into contact with a despoiled item, (for example, tainted water or a dirty contact lens). Bacillus is likewise pathogenic detach; the pathogencity of the gram positive spore framing bacilli for the eye was initially reported in 1980 cause's contamination of cornea additionally Bacillus.

Endophthalmitis is a profoundly unstable disease of the eye that usually brings about quick irritation and vision trouble. In parasitic detachment most normally Aspergillus species were confined from contact lens stockpiling arrangement tests. In Mycotic keratitis Two essential structures have been perceived: that because of filamentous parasites (particularly Fusarium and Aspergillus), these exceedingly pathogenic separates from our specimens may bring about serious eye diseases. Tireless microbial pollution of contact lens stockpiling arrangements is normal and is connected with microbial keratitis and clean corneal penetrates. The absolute most ideal approach to evade eye diseases is to take after appropriate lens care rules as endorsed by the eye care experts. Specifically, including a "rub and flush" stride in the lens cleaning process, minimizing contact with water while wearing contact lenses and supplanting the lens case oftentimes can lessen the danger of disease.

CONCLUSION

We conclude from our research study that the contact lens case solutions are the single most important potential reservoir for contact lens contamination leading to infection. Contact lenses and the solutions used with them are medical devices and are regulated by the Food and Drug Administration; therefore, it is extremely important that patients maintain regular appointments to ensure they are receiving clinical guidance from their eye doctor based on individual eye health needs. Clean and safe handling of contact lenses is one of the most important measures contact lens wearers can take to protect their sight. Exercising optimal care and hygiene with contact lenses can keep the eyes healthy. Our research study found that contact lens wearers who have poor hygiene habits also have increased bacterial contamination in their contact lens cases. Bacterial And fungal contamination was observed in several cases. Colonization of the lens storage case by pathogenic micro-organisms predisposes lens wearers to microbial or sterile keratitis.

Recommendations to contact lens wearers

From this and previous data, the authors suggest the following measures should result in less contact lens case and contact lens solution contamination; thereby possibly reducing the risk

of microbial keratitis, proper use of disinfectant solutions, disinfection of the contact lens case, wash hands before applying lens to the eye, homemade saline never be used, use ophthalmic solution of known high quality brand, and change it regularly.

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