

Reduction of Microbial Contamination along Medical Polymeric Implants

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Abstract: Pathogenic bacteria colonized the mucus coating the tails of intrauterine contraceptive devices (IUCD). This suggests that the IUCD tail may be responsible for the passage of vaginal bacteria into the uterus. Pathogenic bacteria such as *E. coli*, *Staph. epidermidis* and *Bacteroid distasonis* were observed to migrate readily along polymeric fibers (nylon, silk, polypropylene, polyurethane and polyethylene) on the surfaces of nutrient and blood agar. Migration speed was greatest for *E. coli* and slowest for *Bacteroid distasonis*. Nylon was found to support bacterial migration to lowest extent. Antiseptics such as cetrimide, benzalkonium chloride, chlorhexidine, ethylene diamine tetra-acetic acid (EDTA), polyvinyl pyrolidone and sodium dodecyl sulfate (SDS) were tested for efficacy as inhibitors of microbial migration along polymeric fibers. Cetrimide was the best antiseptic used in reduction of microbial migration along the five polymeric fibers.

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1. Introduction

The colonization and adhesion of microorganisms to surfaces of various compositions has been shown to have serious consequences in ecological, medical, and industrial situations (Costerton *et al.*, 1996 and Whitekettle, 2001). Microorganisms can readily attach to a wide variety of surfaces in defined series of steps and in a layering progression that is fairly consistent within a given group of microorganisms (Corpe, 2002). The initial colonizers, frequently specific bacteria, are able to adhere due to their ability to produce exocellular polymers composed primarily of non-ionic and anionic polysaccharides (Costerton *et al.*, 2000, and Dudman, 2004).

Most of medical implants consists of different polymeric fibers such as nylon, silk, polypropylene and polyethylene (Wilkins *et al.*, 2003). Under normal condition the human body is sterile despite the fact that it contains large numbers of many bacterial species. Several workers have proposed that the presence of the medical implants increases the risk of infection (Purrier *et al.*, 2002, Sparks *et al.*, 2004 and Skangalls *et al.*, 2005). Association between the use of medical implants, and increased risk of inflammatory disease has been reported by Gimes, *et al.* (2003) and Ladipo *et al.*, (2005). They postulated that polymeric fibers can act as a pathway for the migration of infectious microorganisms (Mahmoud *et al.*, 2000 and Jayanthi *et al.*, 2008). A number of different bacterial species have been isolated from biofilms adhering to used medical

implants (Marric and Costern, 1999 and Moi, *et al.*, 2001).

The present study has investigated *in vitro* adhesion and migration of most common bacterial species on polymeric fibers of some medical implants and determine the efficacy of a variety of non-toxic surface active compounds as inhibitors of microbial adhesion.

2. Materials and Methods

Bacterial isolates:

Bacterial isolates were obtained from different medical implants such as intrauterine devices (IUCD), medical devices and contact lenses from king khalid hospital in Tabouk city, KSA. They were identified by routine standard technique. Aerobic bacteria were maintained on slants of Brain Heart (BHI) agar (Sigma Chemical Co.) or on nutrient agar. Anaerobic bacteria were maintained on Brewer's anaerobic agar. All cultures were stored at 4 °C. Nutrient broth (aerobic) and egg meat broth (anaerobic) were used for growing liquid bacterial suspensions. Blood agar plates were used for cultivation of *Bacteroides* species in anaerobic environments, nutrient agar plates were used to cultivate aerobic organisms.

Preparation of bacterial suspensions

Aerobic bacteria were inoculated into sterile nutrient broth using cells harvested from an overnight incubation on either brain heart infusion (BHI) or nutrient agar slants (depending on the species used).

After further incubation at 37° C for 24 h, the resulting suspensions were adjusted for their density at 540 nm by spectrophotometer for determination MIC of the used antiseptics against the bacterial isolates. Egg meat broth medium in test tubes was inoculated with anaerobic microorganisms and then placed in anaerobic jar. A mixture of hydrogen and carbon dioxide was generated in the jar by envelopes of activated GasPack (BBL). The jar also contained a catalyst for promoting the room-temperature oxidation of hydrogen to remove oxygen, as well as a control strip for monitoring anaerobic conditions. the jar was incubated at 37 ° C for 24 h.

Fibers:

Five types of polymeric fibers were used, four are synthetic: polyethylene (Hip prosthesis and catheters, joint and ligaments) polypropylene (sutures and plasmapheresis membranes), nylon (polyamide dialysis membranes), polyurethane artificial heart, pacemaker leads heart valves, ventricular assist devices, vascular grafts, dental implants), and one is natural: silk (bioprosthetic heart valve) obtained from Institute of Materials Science, University of Connecticut, USA. The approximate thread diameters are 0.3 mm.

| Polymer | Application |
|---|--|
| Synthetic polymers: Polyethylene polypropylene nylon (polyamide) polyurethane Natural Polymer silk | Hip prosthesis and catheters, joint and ligaments, contact lenses sutures and plasmapheresis membranes dialysis membranes (artificial heart, pacemaker leads heart valves, ventricular assist devices, vascular grafts, dental and orthopaedic implants Bio-prosthetic heart valve |

Antiseptics

Antiseptics used were cetrimide, benzalkonium chloride, chlorhexidine, polyvinyl pyrolidone (PVP), ethylene diamine tetra-acetic acid (EDTA) and sodium dodecyl sulfate (SDS)- which are used in vaginal washer preparations.- from Sigma Chemical Company.

Reduction of microbial migration along polymeric fibers

Aliquots (0.01 ml) of each aerobic microbial suspension were placed on nutrient agar plate and incubated for 24 h at 37 ° C to produce two large colonies. A length (about 3 cm) of polymeric fiber was sterilized in ethanol, dried and placed under aseptic conditions onto the surface of an agar plate so that only one end of the fiber touched a colony while the rest of the fiber resided on sterile agar. Another fiber (3 cm) was pre-soaking in sub-minimal inhibitory concentration of antiseptic for 24 h after overnight soaking in toluene and placed touched to the second large colony. The agar plate was incubated for 24 h at 37 ° C.

In case of anaerobic microorganism, blood agar plates were used and incubated for 72 h at 37 ° C in anaerobic jar. Length of microbial migration along

polymeric fiber and viable count were determined before and after immersion in antiseptics.

3. Results

Vaginal and cervical flora in relation to pre-insertion and post -insertion of copper IUD:

After isolation and purification of vaginal and cervical microorganisms, they identified as: aerobic Gram positive cocci such as *Staph. aureus*, *Staph. epidermidis*, *Streptococci spp* and *Sarcina spp*, Gram negative cocci as *proteus spp* and Gram negative rods as *E. coli*. Anaerobic bacteria such as *Bacteroids distasonis* and *lactobacilli spp*, yeast such as *Candida albicans* were also isolated. Table 1 indicates that aerobic microorganisms as *Staph. aureus*, *Streptococci spp*, *Sarcina spp* and *proteus spp* increased in the percentage after insertion of copper T intrauterine device, but the increase was not significant except with *Staph. epidermidis* and *E. coli* ($P < 0.05$). Anaerobic microorganism such as *Bacteroid distasonis* increased in the percentage after the insertion of the device and the increase was significant, while the increase in yeast such as *candida* was not significant.

Table 1 Vaginal and cervical microorganisms in relation to copper T intrauterine device insertion

| Microorganisms | Vaginal culture | | | | | | Cervical culture | | | | | |
|-------------------------|-----------------|----|----------------|----|----------------|-------|------------------|----|----------------|----|----------------|-------|
| | Pre-insertion | | Post-insertion | | X ² | P | Pre-insertion | | Post-insertion | | X ² | P |
| | No | % | No | % | | | No | % | No | % | | |
| <i>Staph aureus</i> | 4 | 8 | 7 | 14 | 0.9 | NS | 2 | 4 | 2 | 4 | 0.0 | NS |
| <i>S. epidermidis</i> | 2 | 4 | 12 | 24 | 8.3 | <0.05 | 3 | 6 | 10 | 20 | 4.3 | <0.05 |
| <i>Streptococci spp</i> | 3 | 6 | 5 | 10 | 0.5 | NS | 5 | 10 | 5 | 10 | 0.0 | NS |
| <i>Sarcina spp</i> | 1 | 2 | 1 | 2 | 0.0 | NS | 1 | 2 | 1 | 2 | 0.0 | NS |
| <i>E. coli</i> | - | - | 4 | 8 | 5 | <0.05 | - | - | 4 | 8 | 54 | <0.05 |
| <i>Proteus spp</i> | 1 | 2 | - | - | 1.0 | NS | | 2 | - | - | 1.0 | NS |
| <i>Bacteroids spp</i> | 2 | 4 | 8 | 16 | 4.0 | <0.05 | 2 | 4 | 7 | 14 | 4.0 | <0.05 |
| <i>Lactobacilli spp</i> | 20 | 40 | 24 | 48 | 0.7 | NS | 20 | 40 | 23 | 46 | 0.4 | NS |
| <i>Candida spp</i> | 3 | 6 | 3 | 6 | 0.0 | NS | 4 | 8 | 4 | 8 | 0.0 | NS |

Toxicity of antiseptics

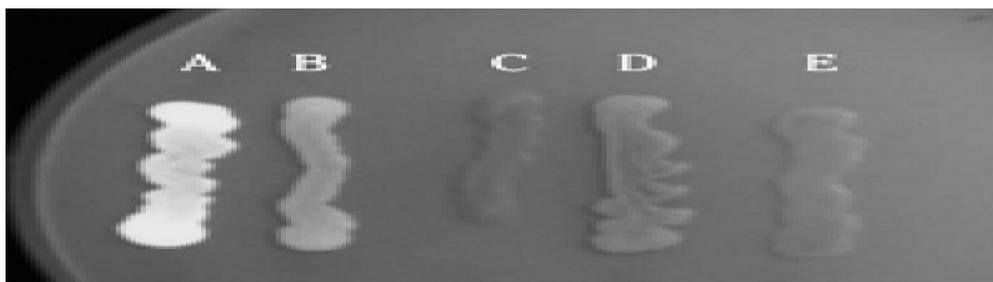
Minimum inhibitory concentrations of different antiseptics were determined against *Staph epidermidis*, *E. coli* and *bacteroid. Distasonis*. Table 1 showed that cetimide, benzalkonium and chlorhexidine were the strongest antiseptics showing the lowest minimum inhibitory concentration. While EDTA, SDS, PVP were the weakest antiseptics.

Table 1 Minimal inhibitory concentrations of different antiseptics.

| Antiseptics | MIC (ug/ml) | | |
|----------------------|----------------|---------------------------|-----------------------------|
| | <i>E. coli</i> | <i>Staph. epidermidis</i> | <i>Bacteroid distasonis</i> |
| Cetrimide | 10 | 20 | 40 |
| Benzalkonium | 250 | 20 | 50 |
| Chlorhexidine | 20 | 30 | 60 |
| EDTA | 100 | 60 | 80 |
| PVP | 140 | 100 | 180 |
| SDS | 450 | 140 | 140 |

Microbial migration along polymeric fibers:

Figure 1 showed microbial migration of *Staph. epidermidis* along polyethylene thread (tail of Cu- T IUCD) on surface of agar plate before immersion in sub MIC of antiseptic solution. The control indicated microbial migration before immersion in antiseptic measured by cm. *Staph. epidermidis* migration along polyethylene fiber (A) = polyurethane (B) were more than polypropylene (D) > silk (E) > nylon (C) before immersion in antiseptic solution (control).

**Figure 1** Migration of *Staph. epidermidis* along polyethylene fiber, polypropylene silk and nylon, polyurethane

After immersion in antiseptic, it was found that cetrimide reduced microbial migration > benzalkonium > chlorhexidine > EDTA > PVP > SDS along the four polymeric used (Figure 2).

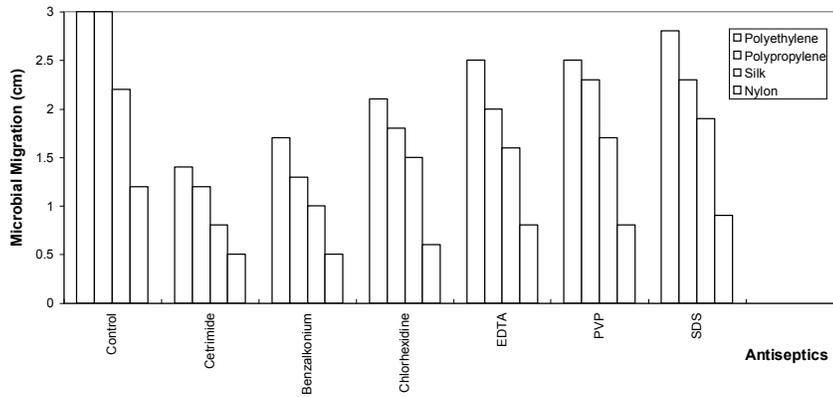


Figure 2 Reduction of *Staph. epidermidis* migration along polymeric fibers using different antiseptics

Figure 3 indicates *E. coli* migration along polymeric fibers before and after immersion in different antiseptics. Cetrimide was the best in reduction of microbial migration, while SDS was the weakest one. Microbial migration along nylon fiber was < silk < polypropylene < polyethylene.

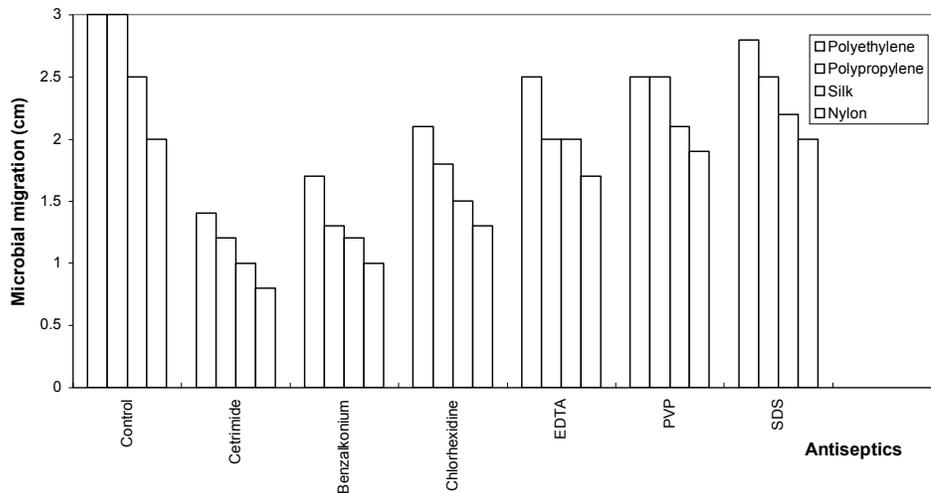


Figure 3 Reduction of *E. coli* migration along polymeric fibers using different antiseptics

Migration speed was slowest for *Bacteroid distasonis* (Figure 4) along nylon fiber and greatest along polyethylene fiber on surface of blood agar plates. Cetrimide showed the highest reduction in microbial migration.

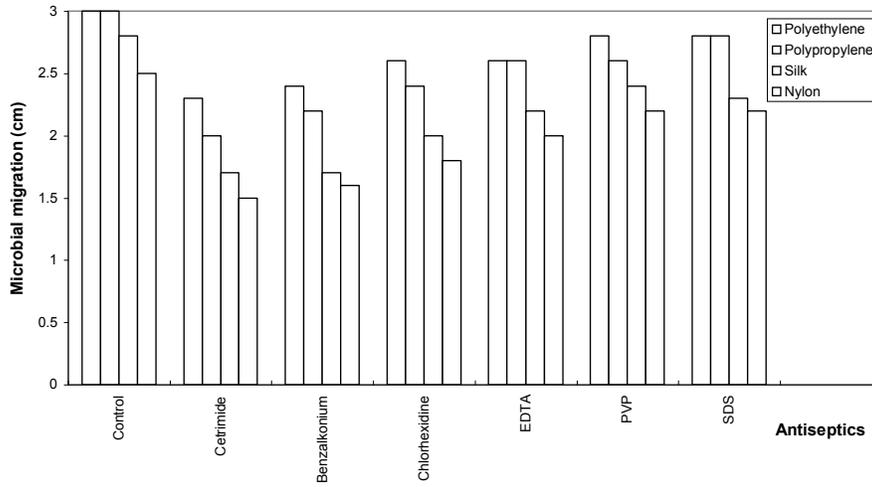


Figure 4 Reduction of *Bacteroid distasonis* migration along polymeric fibers using different antiseptics

Figures 5, 6, 7 indicated that nylon fiber was the best polymeric fiber showing the lowest viable count before and after immersion in antiseptics, Cetimide was the strongest antiseptics and reduction in viable count of *Staph. epidermidis* was > *E. coli* and > *Bacteroid distasonis*.

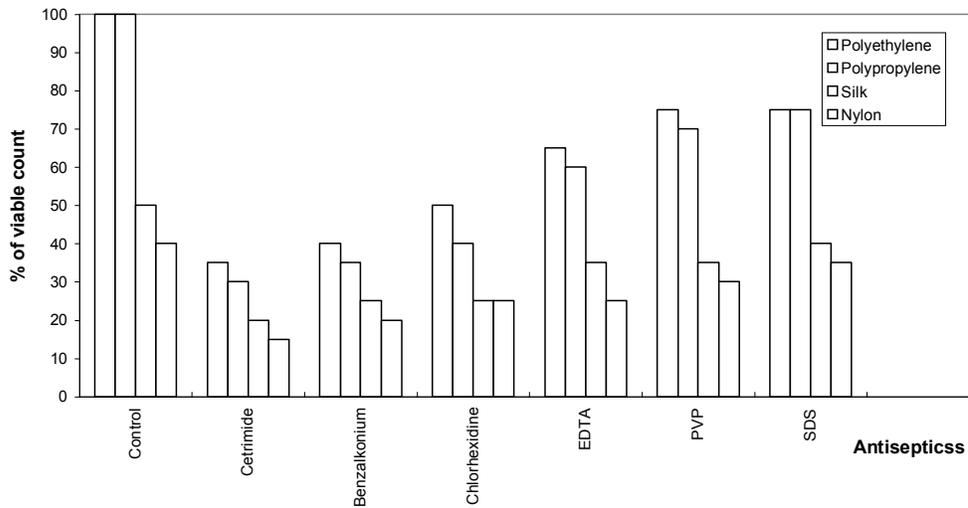


Figure 5 Reduction in viable count of of *Staph epidermidis* using different antiseptics

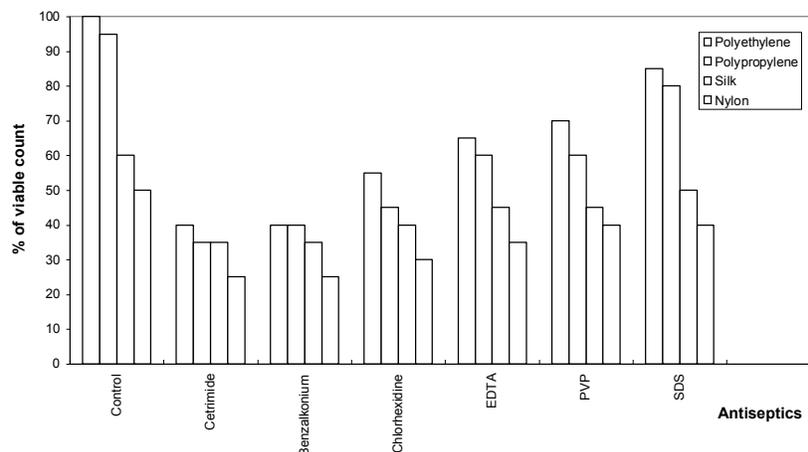


Figure 6 Reduction in viable count of *E. coli* using different antiseptics

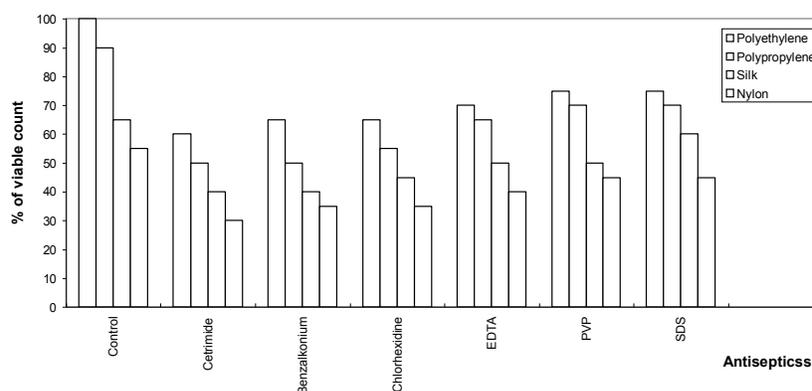


Figure 7 Reduction in viable count of *Bacteroid distasonis* using different antiseptics

4. Discussion

The colonization of medical implants by bacteria is responsible for a great number of implants rejection and failure (Collins, 1994 and Reed and Williams, 2001 Sangit *et al.*, 2012). Bacterial adherence onto the surfaces of intrauterine devices, contact lenses and oral medical implants during their manipulation and insertion is considered to be the initial step of implants related infection such as keratitis associated with contact lens wear or vaginitis after loops insertion implantation (Warner *et al.*, 2003 and Rossi *et al.*, 2007). The present study confirms that potentially pathogenic bacteria will grow along polymeric fibers. In an attempt to fabricate materials that resist bacterial colonization, a variety of compounds known to possess surface-active properties were tested for their ability to prevent the adhesion of microbial populations along different types of polymeric fibers.

Immersion fibers in sub-minimal inhibitory concentration of different antiseptic for 24 h after overnight immersion in toluene will reduce the microbial migration to some extent. In our results, the greatest efficacy of cetrimide as inhibitor for microbial adhesion on polymeric fibers may be due to its highest ability as surfactant to lower surface tension of the aqueous environment beside to its potency as antiseptic.

On immersion thread in toluene overnight, the adsorption of macromolecules increased pore size of polymeric fibers and thus adsorption capacity to antiseptic. Such adsorbed molecular layers are termed conditioning films, because they alter surface charge and surface free energy (Baier, 2003, Fletcher, Marshall, 2004 and Holá *et al.*, 2006) of the substratum.

Conditioning films in nature play a role in modifying the extent of bacterial adhesion to

immersed surfaces. The extent of bacterial transmission along polymeric fibers appeared to be primarily determined by the motility of the organism. Motile organism (*E. coli*) was found to progress to greater extent than non-motile (*Staph. epidermidis* and *Bacteroids*) even after treated fibers by antiseptics. It is proposed that motile bacteria can easily pass along the thread surface (Wilkins *et al.*, 2003). These observations concur with our results that showed that the bacteria had adhered to, and migrated along the fiber leaving the upper part of fiber treated with antiseptic. Migration was fastest for *E.coli*, slowest for *B. distasonis* and of intermediate speed for *Staph. epidermidis*.

The polymeric fibers used are normally hydrophobic, microorganisms excrete surface active compounds (fatty acids) that can change such surfaces from hydrophobic to hydrophilic, at least to some significant extent. Microorganisms also excrete exopolysaccharide adhere to the surface of polymeric fiber. Thus the migration observed along control fibers due to swimming motility of bacteria through a microconduit of water capillary -condensed between fiber and agar (Costeron *et al.*, 2000 and Rao *et al.*, 2008).

The lowest microbial migration and adhesion along nylon may be due to high molecular weight of nylon that means a less hydrophilic surface, more crystallinity and less enzyme substrate interaction and (Klemchuk, 1990 and Nishida, 2002 Eftekhar F. and Z. Mirmohamadi, 2009). The results documented the use of nylon (high molecular polymeric fiber) instead of polyethylene and pre-immersion or coating them with antiseptic acting as surfactant (cetrimide) to decrease microbial adhesion, migration and biofilm formation along polymeric fibers

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