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Effect of Anti-Microbial Fiber and its Interaction with Penicillin G on Opportunistic Skin Micro-Flora

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Abstract

Objective
The standard of hygiene in daily life and hospitals can be increased by the use of new antimicrobial fibers, which diminish the danger of pathogenic bacteria. In this study, the antimicrobial effect of special fibers on some staphylococcus isolates was investigated.

Materials and Methods
The antimicrobial effect of special type of fibers produced in Isfahan Poly Acryl Plant on three species of *Staphylococcus aureus*, *epidermidis* and *lugdunensis* isolated from 96 samples of hand and foot skin micro-flora was studied. The sensitivity of strains regarding resistant strains, to various antibiotics and beta-lactamase enzyme production was studied. The most resistance to antibiotics and beta-lactamase producer were chosen. Using the cup plate method, the inhibiting effect of pure antimicrobial agent on these strains was proven. Next, using shake flask method the effect of antimicrobial fiber on these strains was studied. In order to compare the effect of the antimicrobial agent of the fiber with that of penicillin G, the minimal inhibitory concentration (MIC) of the fiber antimicrobial agent and of penicillin G was tested on the strains. The effect of the interaction of these two antimicrobial agents and their fractional inhibitory concentration (FIC) on the chosen strains was studied using checkerboard method.

Results
The results show a significant effect by antimicrobial fiber with 30%, 60% and 100% antimicrobial agent on *Staphylococcus* species after 24 hrs. Moreover despite the high level MIC of penicillin G on these bacteria (8-256 µg/ml), the MIC of the pure antimicrobial agent of fiber at a level of 10^{-4} µl/ml caused growth inhibition. The interaction of these two antibacterial agents on the chosen strains was evaluated as synergism.

Conclusion
According to this study the antimicrobial effect of the fiber on growth inhibition of common, resistant skin bacterial flora is positive and therefore may be used after other successful clinical trials.

Keywords: Antimicrobial fibers, Fractional inhibitory concentration (FIC), Minimal inhibitory concentration (MIC), Skin micro-flora

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Introduction

Due to the wide spread and important role of textiles in everyday life and considering the fact that textiles, especially when combined with factors such as heat, humidity, dust, soil and fat-stains (on the surface), are one of the best and most common environments for the growth and distribution of microorganisms, which endanger hygiene and also create unpleasant odors (1) and considering the variety of microbes living on human skin and the increase in their resistance to drugs, recently, consistent with nanotechnology progress attempts at modifying fibers based on the biochemical mechanism of disease and the molecular design to create high performance health care textiles have been made and have revolutionized the medical science (2). The fact is that the use of textile engineering for improvement of fibers which have contact with human skin has a very long history: 5000 years B. C. Linen clothes containing salt was used to wrap mummies, also in 1867 Joseph Lister used bandages soaked in carbolic acid and phenol in surgery as antiseptic (which seems to be the first scientific attempt in this regard) (1). But due to fast progress, new antimicrobial agents and improved polymer substances such as copper and silver compounds, chitosan, triclosan, quaternary ammonium compounds, polymeric phosphonium salts, polymeric biguanides and N-halamin compounds were developed and were added, during or after the spinning process, to fiber under closely controlled conditions (3, 4).

Along this line of development, Isfahan Poly Acryl Plant with the cooperation of textile engineers and with the aid of chemical technologies and biotechnology has recently manufactured acrylic fiber enriched with tertiary ammonium molecules (selected because of porous structure of acrylic fiber and size of these pores), which have antimicrobial properties and can control the growth of some types of bacteria and fungi. In this study the antimicrobial effect of this fiber was determined on three common species of skin flora staphylococci i.e. *Staphylococcus aureus*, *S. epidermidis* and *S. lugdunensis* with multiple antibiotic resistance, and also on a strain of *S. aureus* (ATCC 6538p = PTCC 1112) obtained from Persian type culture, Collection of Iran, Iranian Research Organization for Science and Technology, Tehran, Iran.

Materials and Methods

Sampling and laboratory technique

Skin sampling was carried out using a sterile swab soaked in trypticase soy broth (TSB, Himedia, India). The swab was rubbed three times in one direction over a predetermined length (3 cm) on the target area of the hand or foot, then put in a test tube containing 5 ml sterile TSB, transported to the laboratory and incubated at 37 ºC for 24 hrs. For bacteria identification, gram staining and laboratory biochemical tests were utilized (5).

Antimicrobial susceptibility testing

Determination of β-lactamase activity

In order to study the mechanism of resistance to β-lactam antibiotics, β-lactamase presence in these bacteria was determined by acidimetric method. For this purpose 2 ml of aqueous phenol solution (0.5%) was diluted with 16.6 ml of distilled water, and 1.2 g of penicillin was added. The test reagent was adjusted to pH 8.5 with 1 mol/L NaOH giving violet color to the solution. Then the end of a capillary tube (0.2-1 mm diameter) containing freshly made test reagent was put on the target colony of the bacteria grown on nutrient agar for 24 hrs. The appearance of a yellow color in 5-15 mins indicates β-lactamase activity (6).

Disk diffusion method

The Kirby-Bauer disk diffusion method (5) was used to test the antibiotic susceptibilities of *Staphylococcus* strains isolated from skin flora using Muller-Hinton agar (MHA, Merck, Germany) and disks containing penicillin G (10 IU)., ampicillin (10 μg), amoxycillin (25 μg), cloxacillin (5 μg), cephalothin (30 μg), cephalexin (30 μg), cephotaxime (30 μg), ceftazidime (30 μg), ceftriaxon (30 μg), ceftizoxim (30 μg), tetracycline (30 μg), tobramicine (10 μg), amikacin (30 μg), gentamicin (10 μg), vancomycin (30 μg), trimethoprim (1.25 μg), sulphamethoxazole (23.75 μg) (Padtan Teb Company, Iran).

Cup plate method

The antimicrobial activity of the pure antimicrobial agent of antimicrobial fiber against *Staphylococcus* strains was tested using cup-plate method. In this method sterilized petri plates were prepared with an equal thickness of MHA. Test organism was grown at 37 ºC in Muller-Hinton broth to achieve a turbidity equivalent to a 0.5 MacFarland standard (approximately 1-1.5×10^8 organisms/ml). This broth culture was used for seeding the agar plates. Then cups with 6 mm in diameter were created in seeded agar with appropriate spaces (2 cm) (7). Ten µl, 50 µl and 100 µl of the pure antimicrobial agent were added to each cup respectively, then plates were placed in 4 ºC for 1-2 hrs and finally after 24 hrs incubation at 37 ºC, the zones of inhibition were measured (1, 5). All the experiments were performed in duplicate and results were interpreted by analysis of variance method.

Antibacterial activity of fiber

The antibacterial properties of the normal fibers and the treated fibers with different percentages of antimicrobial agent (30, 60 and 100%) were evaluated by ASTM E2149-01 (American Society for Testing and Materials, 2001), which is a quantitative antimicrobial test method preformed under dynamic contact conditions. The choice of this method is connected to the hydrophobic nature of polyester fiber and to the additive activity mechanism. In the operating procedure 0.5 ml of working bacterial inoculums with concentration 1-1.5×10^8 colony forming unit (CFU)/ml was added to a 100 ml Erlenmeyer flask containing 49.5 ml of phosphate buffer and 0.5 g of the test specimen of fiber. After sampling at time zero, a series of dilutions of the bacterial solution using the buffer solution from each flask was prepared, 5 drop containing 0.1 ml of the suitable dilution were plated in nutrient agar (Drop-plate method) (8). The inoculated plates were incubated at 37 ºC for 24 hrs and surviving cells were counted (it should be noted that in terms of statistics, 3-30 colonies in each drop is acceptable). The average values were converted to CFU/ml in the flasks by multiplying by the dilution factor. The same procedure was repeated after all flasks were capped loosely and placed on the incubator, and shaken 85-95 rpm for 24 hrs at 37 ºC (Unimax model 20.0 Heidolph). The counting was done in triplicate each time (7, 9). A flask containing test bacterium and buffer solution as a blank was kept and counted with each experiment in order to determine the growth rate of the bacterium in buffer solution.

The antimicrobial activity was expressed in terms of % reduction of the organism after contact with the test specimen compared to the number of bacterial cells at time zero. The percentage reduction was calculated using the following equation,

$$\%\text{Reduction}=\frac{B-A}{B}\times 100$$

where A and B are the surviving cells (CFU/ml) after 24 hrs and at time zero, respectively (7). All the experiments were performed in triplicate and the results are displayed as the mean value of the experiments. Results were studied using analysis of variance method.

MIC determination

The minimal inhibitory concentration (MIC) of antimicrobial agent of the test fibers on target bacteria was determined by a standard agar dilution method. In this method each examined dilution of antimicrobial agent (1 ml) (dilution levels of 4×10^{-4}, 5×10^{-4}, 6×10^{-4}, 7×10^{-4}, 8×10^{-4}, 9×10^{-4}, 1×10^{-3}, 2×10^{-3}, 3×10^{-3}, 4×10^{-3} µl/ml) was incorporated into a single agar plate (containing 24 ml sterile molten MHA), plus one positive growth control plate without antimicrobial agent. The surface of each plate was inoculated with 1×10^4 CFU/spot. Plates were incubated at 37 ºC for 24 hrs and then examined. The MIC was defined as the lowest concentration of antimicrobial agent that yielded no growth or marked change in the appearance of growth compared to the growth control plate (5).

Broth micro dilution method was used for determining the minimal inhibitory concentration (MIC) of antibiotic treatment (penicillin G) on target bacteria. Bacterial suspensions (5×10^5 CFU/ml) were prepared from a fresh, overnight

and pure culture of test bacterium were incubated for 24 hrs at 37 ºC in microtitre tray in the presence of series of doubling two fold dilutions of penicillin G in each microtitre well. Each tray included a growth control that was not contained antimicrobial agent and sterility control that did not inoculated. Therefore microtitre wells from 1-12 contained 0, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, and 2048 µg/ml of penicillin G, respectively. Following incubation, the micro dilution tray was examined for bacterial growth. The growth pattern in micro dilution wells was detected and interpreted by optical reading the amount of light transmitted through each well with the ELISA reader system (Statfax-2100, Awareness Technology Inc., USA) (5).

Following determination of penicillin G MIC, its minimal bactericidal concentration (MBC) on examined bacteria was also determined by incubation an aliquot from each well in the dilution series, showing inhibition of bacterial growth on MHA at 37 ºC for 48-72 hrs (5). Finally the lowest level of antibiotic being able to kill 99.9% of the bacteria was determined as MBC. All the experiments were performed in duplicate and the results were displayed as the mean value of the experiments. The MIC of the two antimicrobial agents was compared by ANOVA statistic test.

Fractional inhibitory concentration (FIC) determination and interaction effect of two antimicrobial agents (pure antimicrobial agent of fibers + penicillin G) on test bacteria

To assess antimicrobial combinations *in vitro* the checkerboard method was selected. In this technique by using agar dilution method, the concentrations tested for each antimicrobial agent were typically ranged from four or five below the expected MIC to twice the anticipated MIC as in the 45 degree line in Figure 1 (each square represents one plate), using two fold dilutions of each antimicrobial agent, also concentration of MIC point and a dilution lower than it for each antimicrobial agent alone. Then 1 ml of predetermine dilutions of the two antimicrobial agents were added to sterile and molten Muller-Hinton agar (with a temperature of 50-55 ºC) (23 ml) and, after mixing, deposited on sterile plate. Then the surface of each plate was inoculated with 1×10^4 CFU/spot of bacteria. After 16-20 hrs incubation at 37 ºC, the plates were examined for evidence of visible growth (10).

The FIC index was then calculated by using the following equation by summing the separate FICs for each of the drugs present in that plate:

$$FIC_{index} = FIC_A + FIC_B = \frac{(A)}{(MIC_A)} + \frac{(B)}{(MIC_B)}$$

where A is the concentration of drug A in a plate that is the lowest inhibitory concentration in its row, MIC_A is the MIC of the organism to drug A alone, and FIC_A is the fractional inhibitory concentration of drug A. Also B is the concentration of drug B in a plate that is the lowest inhibitory concentration in its column, MIC_B and FIC_B are defined in the same fashion for drug B.

With this method, synergism has traditionally been defined as an FIC index of 0.5 or less and additivity as a FIC index of 1.0; antagonism has been defined as a FIC index of 2.0 (10).

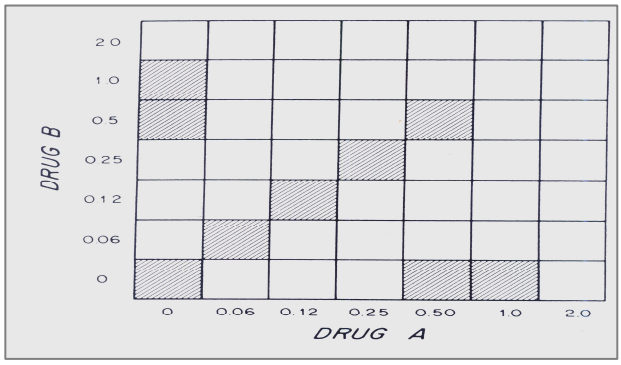

Figure 1. Simplified checkerboard method (MIC A and B drugs is considered as 1µg/ml) (10).

Results

In this research, in 96 samples from hand and foot surfaces, 180 bacterial strains were isolated and identified by microbiologic standard methods (5). Then one strain of *S. aureus*, one strain of *S. epidermidis* and one strain of *S. lugdunensis* were selected by using two screening stages (β-lactamase enzyme presence and profile of antibiotic resistance). Overall; all types of examined staphylococci produced β-lactamase and then were resistant to the most of β-lactam antibiotics (Table 1).

Table 1. Antimicrobial susceptibility pattern of examined bacteria by disk diffusion method and determination of β-lactamase activity.

Bacteria	β-lactamase	Trimethoprim-Sulphamethoxazole	Vancomycin	Gentamicin	Amikacin	Tobramicin	Tetracycline	Ceftizoxim	Ceftriaxon	Ceftazidime	Cefotaxime	Cephalexin	Cephalothin	Cologsacillin	Amoxycillin	Ampicillin	Penicillin
S. aureus	+	S	R	R	S	S	R	S	I	R	I	I	S	R	-	R	R
S. epidermidis	+	S	S	-	S	-	-	I	I	R	I	S	S	R	R	R	R
S. lugdunensis	+	S	S	S	S	S	S	s	S	-	S	S	S	R	R	R	R

Table symbols: R: Resistance, S: Sensitive, I: Intermediate, -: not determined, +: Production of β-lactamase (according to Padtan Teb standards).

Using cup plate method, the inhibition zone around cups of pure antimicrobial agent were more than 20 mm. i.e. Susceptibility of the resistant Staphylococci to different levels of pure antimicrobial agent of the test fiber is high.

The antibacterial properties of the selected fiber were evaluated by shake flask method, because the antimicrobial efficacy of a compound will vary when it is held intimately by a textile substrate.

The results in Table 2 show that fibers containing various levels of quaternary ammonium compounds have significant antibacterial activity. The results of this study on MIC are been shown in Table 3.

Table 2. Average change in resistant skin's flora growth based on duration of contact with antimicrobial fiber.

Bacteria	Fabrics with 100% antibacterial agent	Fabrics with 60% antibacterial agent	Fabrics with 30% antibacterial agent	Normal fabrics	Blank	Time of sampling (hrs)	P value
S. aureus	1.3×10^5	1.6×10^5	1.2×10^5	1.5×10^5	1.4×10^5	0	
	1.5×10^4	4.1×10^4	1.4×10^5	4.3×10^5	2.7×10^5	6	
	1×10^4	1.2×10^4	1×10^4	4.2×10^5	1.9×10^6	24	
	-92	-92	-92	+180	+1257	differences%	
S. epidermidis	1.4×10^4	1.2×10^4	1.6×10^4	1.2×10^4	1×10^4	0	<0.001
	0	0	0	1.8×10^4	1.6×10^4	6	
	0	0	0	1.2×10^5	1.9×10^5	24	
	-100	-100	-100	+900	+1800	differences%	
S. lugdunensis	2.1×10^5	1.6×10^5	3.2×10^5	1.6×10^5	1.6×10^5	0	<0.001
	0	0	0	1.64×10^5	1.6×10^5	6	
	0	0	0	1.8×10^5	2.7×10^5	24	
	-100	-100	-100	+11	+68	differences%	
S. aureus (ATCC 6538p)	1.5×10^4	1.57×10^4	1.76×10^4	1.6×10^4	1.45×10^4	0	
	0	0	3.85×10^3	3.8×10^3	5.1×10^4	6	
	0	0	0.11×10^3	3.4×10^3	7.05×10^4	24	
	-100	-100	-99.37	-79.01	+79.4	differences%	
+: bacterial increase, -: bacterial reduction							<0.001

Table 3. MIC values of the examined quaternary ammonium compound and penicillin G on selected staphylococci.

P value (MIC)	Penicillin G (µg/ml)		Pure antibacterial agent of fiber(µl/ml)	Bacteria
	MBC	MIC	MIC	
<0.001	512	256	0.0007	***S. aureus***
<0.001	512	128	0.002	***S. epidermidis***
<0.001	16	8	0.0007	***S. lugdunensis***
<0.001	0.25	0.03	0.0007	***S. aureus (ATCC 6538p)***

Pure antimicrobial agent of fiber was very active, with an MIC, at which all strains were inhibited (MIC_{90}), of $\leq 2\times10^{-3}$ µl/ml; while these strains required 8-256 µg/ml of penicillin G for inhibition. Thus there is substantial difference between pure antimicrobial agent MIC and penicillin G MIC in all strains (P<0.001) indicating more inhibitory effect of pure antimicrobial agent on antibiotic treatment of staphylococci.

The FIC level and the interaction of the two antibacterial agents demonstrated the synergic effect on all three strains of *Staphylococcus* (Table 4).

Table 4. FIC and interaction examined quaternary ammonium compound with penicillin G on selected staphylococci.

Bacteria	FIC_{index}	FIC_B	FIC_A	Reaction
S. aureus	0.31	0.06	0.25	synergism
S. epidermidis	0.31	0.06	0.25	synergism
S. lugdunensis	0.31	0.06	0.25	synergism
S. aureus (ATCC 6538p)	1.16	0.5	0.66	additivity

A: quaternary ammonium compound, B: penicillin G

Discussion

Microbial growth control in textiles is very important because of hygiene and increase in fiber duration which decreases due to biological disintegration. The use of fiber with antimicrobial properties can play a significant role in satisfying of these goals.

As shown by the results of this study, which reflects the significant effect of test fiber on the three species of the most common skin flora, tertiary ammonium compound used as antimicrobial agent in acrylic fiber even in its least amount (10 µl) can significantly inhibit the growth of tested *Staphylococcus* strains resistant to most related antibiotics.

Analysis of variance for Table 2 data shows substantial effect of fiber containing various levels of antimicrobial agent in decreasing the number of bacteria in all four strains after 24 hrs in comparison with the blank and normal fabrics (P<0.001). The effect on the tested *S. epidermidis* and *S. lugdunensis* was 100% decrease and >90% decrease in *S. aureus*.

In order to be sure of the results and to determine the probable mistakes and other factors which may not have been taken into consideration, the effect of antimicrobial fiber on the standard strain of *S. aureus* (ATCC 6538p) was tested under similar conditions. Comparison of these results to those of test strains isolated from skin flora revealed that there was no substantial difference in all examined groups except in the case of the normal fiber group. This shows the effective antimicrobial property of the test fiber against standard type and harmful Gram-positive bacteria tested in this study.

In comparison with the present results, Yao recorded antibacterial efficiency of the microporous polypropylene hollow fiber membranes with surface grafted block copolymer brushes of poly (ethylene glycol) monomethacrylate and 2-(dimethylamino) ethyl methacrylate on *S. aureus* being 99.99% after 6 hrs contact (11). According to Chen Yao study in 2008, antibacterial efficacy of surface modified electrospun polyurethane fibrous membranes with quaternary ammonium moieties was 99.9% for *S. aureus* after 1 hr contact (12). Shin, *et al* reported that using Polypropylene nonwoven fabrics treated with chitosan oligomer solution (at 0.01% and 0.05% levels) decreased *S.aureus* population >90% (13). Salvio et al reported the reduction of *S. epidermidis* (ATCC 12228) treated with antimicrobial fiber under the trade mark name of Tertial Saniwear as being 97% (14). White *et al* reported that antimicrobial fiber containing AEM 5700 (trade name) antimicrobial agent caused reduction 99% and 96% in *S. aureus* and *S. epidermidis* isolated from foot, respectively (15). Also, according to Ye's experiments in 2005 using cotton fabrics coated with 300 nm novel core-shell particles (that consist of poly (n-butyl acrylate) cores and chitosan shells) inhibited *S. aureus* (ATCC 6538p) growth by >99% (16). And finally, Dubas recorded nylon and silk fiber coated with silver nanoparticles as affecting a

strain of *S. aureus* by 53% and 80%, respectively (17).

The difference seen in the above decrease percentages could be accorded to the test bacteria strain tested, the type of fiber, antimicrobial agent and its concentration in fiber and assay conditions such as growth medium, the length of time for treatment of bacteria and the original number of bacteria. However, it seems all the Gram-positive bacteria wed in the reports, especially the present report, are substantially sensitive to antimicrobial fibers.

So in comparison with the study findings about pure antimicrobial agent of fiber MIC (Table 3), Shao, *et al* in 2003 reported that the MIC of perfluoroalkyl antimicrobial agent (used in providing antimicrobial fibers) was 7.8 µg/ml for *S. aureus* (ATCC 6538) (18) and according to Hayes *et al* in 2005 using pure antimicrobial agent of fiber under the trade mark name of AEM 5700 was 10 µg/ml for *S. aureus* (19).

Although pure antimicrobial agent of fiber was very effective on bacteria; regarding to Table 2 antimicrobial efficacy of this compound is lower but really obvious when it is held intimately by the textile substrate on selected bacteria.

Doern et al reported on penicillin G MIC on *S. aureus* strains isolated from skin and soft-tissue infections was ≤0.015>32 µg/ml (20). Fass et al in 1986 recorded MIC of penicillin G on *S. aureus* and *S. epidermidis* producing β-lactamase were isolated from clinical specimens >0.25 µg/ml (21) and Goldstain recorded penicillin G MIC on *S. aureus* strains isolated from bite wound infections ≤0.015-8 µg/ml (22).

Comparing the fiber antimicrobial agent MIC with MIC and MBC penicillin G, which is prescribed as the first selected antibiotic for treatment of staphylococcal infections, (Table 3) shows: (1) widespread resistance of skin flora to antibiotics and (2) effectively powerful antimicrobial property of pure antimicrobial agent of fiber when considered as a bactericidal factor in growth inhibition of bacteria compared to penicillin G.

Another significant issue in this study was the synergistic effect of compounding the two antimicrobial agents (the pure antibacterial agent of fibers plus penicillin G) tested on staphylococci skin flora. This could be explained by destruction of cytoplasmic membrane by tertiary ammonium compounds, and stopping the synthesis of β-lactamase, though multiple site of cell targeted by pure antimicrobial agent of fiber and penicillin G.

Conclusion

The present research confirms the antimicrobial activity of studied fiber. After successful clinical trials this antimicrobial textiles can satisfy a significant role in growth control of resistant staphylococci the skin flora.

It can be stated, with regard to the present study, that one of the best methods which can inhibit growth of microorganisms (consisting of bacteria, fungi, algae etc.) and diminish their harmful effects is the use of antimicrobial fiber as a technology with ever increasing importance, in all environments (e.g. households, work places, stadiums, hospitals).

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References

1. Vincent EJ, Vigo TL. Bioactive fibers and polymers. Washington, D. C.: American Chemical Society; 2001.
2. Tew GN, Liu D, Chen B, Doerkson RJ, Kaplan J Carroll PJ, *et al*. De novo design of biomimetic antimicrobial polymers. PNAS 2002; 99:5110-5114.
3. Perepelkin KE. Principles and methods of modification of fibers and fiber material. Princ Fib Chem 2005; 37:123-140.
4. Worley SD, Sun G. Biocidal Polymers. Trends Polymer Sci 1996; 4:364-370.

5. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scotts diagnostic microbiology. Missouri: Mosby Inc; 2002.
6. Rice LB, Bonomo RA. Genetic and biochemical mechanisms of bacterial resistance to antimicrobial agents. In: Lorian V. editor. Antibiotic in laboratory medicine. Philadelphia: Williams & Wilkins; 2005. p.486.
7. Knill CJ, Kennedy JF, Mistry J, Miraftab M, Smart G, Groocock MR, Williams HJ. Alginate fibres modified with unhydrolysed and hydrolysed chitosan for wound dressings. Carbohyd Poly 2004; 55:65-76.
8. Pongsamart S, Nanatawanit N, Lertchaipon J, Lipipun V. Novel water soluble antibacterial dressing of durian polysaccharide gel. In: III WOCMAP Congress on Medicinal and Aromatic Plants-Volum4: Targeted Screening of Medicinal and Aromatic Plants, Economics and Low; Chiang Mai 2005; Thailand.
9. Renaud FN, Freney J. Les textiles antimicrobiens. Pour to science 1999; 266.
10. Pillai SK, Moellering C, Elipoulos GM. Antimicrobial combinations. In: Lorian V. editor. Antibiotic in laboratory medicine. Philadelphia: Williams & Wilkins; 2005. p. 365-373.
11. Yao F, Fu G D, Zhao J, Kang ET, Neoh KG. Antibacterial effect of surface-functionalized polypropylene hollow fiber membrane from surface-initiated atom transfer radical polymerization. J Membr Sci 2008; Article in press.
12. Yao C, Li X, Neoh KG, Shi Z, Kang ET. Surface modification and antibacterial activity of electrospun polyurethane fibrouse membranes with quaternary ammonium moieties. J Membr Sci 2008; Article in press.
13. Shin Y, Yoo D, Min K. Antimicrobial finishing of polypropylene nonwoven fabric by treatment with chitosan olygomer. J App Polym Sci 1999; 74:2911-2916.
14. Salvio G. A new polyester fiber with antibacterial activity. MONTEFIBRE SpA 1999; Available: http://www.montefibre.it/en/polyester/pdf/sani_con00.pdf.
15. White WC. A new, durable antimicrobial finish for textile. ÆGIS Microbe Shield. 2005; Available: http://www.aegisasia.com/antimicrobialfinish.html
16. Ye W, Leung MF, Xin J, Kwony TL, Lee DL. Novel core-Shell particles with poly (n-butyl aerylate) cores and chitosan shells as an antibacterial coating for textiles. Polymer 2005; 46:10538-10543.
17. Dubas ST, Kumlangdudsana P, Potiyaraj P. Layer-by-layer deposition of antimicrobial silver nanoparticles on textile fibers. Colloids and Surfaces 2006; 289:105-109.
18. Shao H, Jiang L, Meng WD, Qing FL. Synthesis and antimicrobial activity of a perflouroalkyl-containing quaternary ammonium salt. J Fluorine Chem 2003; 124:89-91.
19. Hayes SF, White WC. How antimicrobial treatment can improve nonwovens. 2005. Available: http://www.aegisasia.com/improvenonwovens.html
20. Doern CV, Jones RN, Pfaller MA, Kugler KC, Beach ML. The SENTRY Study Group (North America). Bacterial pathogen isolated from patients with skin and soft tissue infections: Frequency of occurrence and antimicrobial susceptibility patterns from the SENTRY antimicrobial surveillance program (United State and Canada, 1997). Diagnos Microbiol Infect Disease 1999; 34:65-72.
21. Fass RJ, Helsel VL, Banishan J, Ayers LW. *In vitro* susceptibilities of four species of coagulase negative Staphylococci. Antimicrob Agent Chemother 1986; 30:545-552.
22. Goldstein EJC, Citron DM, Merriam CV, Warren Y, Tyrrell K. Comparative in vitro activities of GAR-936 against aerobic and an aerobic animal and human bite wound pathogens. Antimicrob Agent Chemother 2000; 44:2747-2751.