ORIGINAL RESEARCH PAPER

Volume-7 | Issue-11 | November-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ANTIMICROBIAL ACTIVITY OF ALCOHOLIC GEL AGAINST BACTERIAL FLORA-PRODUCING MALODOR ISOLATED FROM FOOT AND AXILLARY REGION

Biological Science

Zainab A . M. Al-Tamimi Assisstant Lecturer, College of Education -AL Muqdad

Dr. Majid M. M. Al-Jewary* Prof, College of Science -Mustansiriya University *Corresponding Author

Dr. Dr. Abbas A. F. AL-Dulaimi Prof, College of Education for Pure Science

ABSTRACT

This study was conducted to evaluate antimicrobial activity of alcoholic gel on malodor-producing bacterial flora which were clinically isolated from foot and axilla of individuals at various age groups. This work was performed during the period from April 2012 to July 2012. One hundred and sixteen swabs from foot and axilla -of male and female- were collected at Al-Muqdadiyah General Hospital, Diyala. The control group included 60 swabs were collected from apparently healthy individuals. The swabs of foot and axilla were inoculated onto suitable culture media and, then bacterial cells were identified based on morphological and biochemical characteristics. After application of alcoholic gel (mL/L), foot and axillary swabs were re-taken again and inoculated every (2,4,6,10) hours in order to identify the time point at which the gel shows its highest antibacterial activity. Our data showed that *staphylococcus epidermidis* was the primarily isolated bacteria followed by *Corynebacterium spp.* From the total samples, the number of bacteria isolated from axilla were as following; *Staphylococcus epidermidis* 77 (46%), *Corynebacterium spp* 50 (30%), *Staphylococcus aureus* 37 (22%). From foot region, the isolated bacterial cells were *S.epidermis* 9 (39%), *corynebacterium spp* 8 (34%), and *S.aureus* 6 (26%). In addition, we reported that alcoholic gel had an inhibitory effect against isolated bacteria only during the first 2 hours of treatment. However, bacterial cells were capable to re-grow multiply and produce malodor after the 2 hours-treatment are ended. Comparing to several deodorants, alcoholic gel is highly recommended as safe and suitable treatment of choice for body malodors.

KEYWORDS

Bacterial normal flora, Foot and axilla, Antimicrobial effect, Alcoholic gel

INTRODUCTION

Sweating, the natural physiological phenomenon, works as regulator of our body temperature by releasing amounts of sweat controlled by the nervous system. This phenomenon is affected by the type of climate (hot weather), patient's physical status (exercise, anger, fear), pathological conditions accompanied with fever, or even, eating spicy foods (1). The "sweating system" composed of about 3 millions functional units called the sweat glands. At the foot, there are about 500000 glands and the others, 2500000, are distributed at three regions; the lips, teats and the skin of genital organs. At the crowded area, secretion and smelling of sweat upsets a lot of individuals who wearing non-cotton clothes that prevent natural evaporation of sweat (2).

Naturally, body smell of foot and axilla comes from biochemical reactions of bacterial flora, especially *Corynebacterium* spp with sweat's fatty substances and proteins (3). Several people are used to apply deodorants which are not enough to reduce sweat secretion itself. Some others are using aluminum-containing antiperspirants which block the sweat gland duct and ceases sweat secretion. However, many recent studies refer to the possibility of having cancer or aluminum poisoning due to using such antiperspirants (4).

Poisoning with aluminum and it's harmful effect on mental activities are still a controversial issue while not a single scientific report has proved to have cancer because of using aluminum preparations (5).

The medical, social, and economic impacts of sweating secretion and its malodor should attract the attention of scientists to find ideal alternatives for controlling such phenomenon. Few studies have been published about this topic. Therefore, the current work comes to shed the light on the following; (i) detection of bacterial normal flora of foot and axilla and bacterial causes of malodor, (ii) sensitivity of isolated bacteria to alcoholic gel in order to find a safe substances as antiperspirants instead of using synthetic chemicals.

MATERIALS AND METHODS

This study included collection of 116 foot and axilla swabs from individuals (56 male, 60 female) having malodor sweating at Al-Muqdadiyah General Hospital, Diyala. After swabs were taken, they inoculated directly on blood agar, manitol salt agar and tellurite agar. This study included a control group, 60 swabs were taken from apparently healthy individuals. Blood agar and manitol agar plates were incubated aerobically at 37°C for 24-48 hr while tellurite plates were kept at same conditions for 48-72 hr (6). After applied of 2 ml of alcoholic gel **(1mL/L)** and left for 30 sec to dry, foot and axillary swabs were re-taken again and inoculated every (2,4,6,10) onto same above mentioned culture media and at same conditions. Bacterial cells were identified according to (7,8,9) based on the morphological and biochemical features.

RESULTS AND DISCUSSION

1-Samples collection

In this study, 116 samples were collected from healthy people undergo from foot and axilla malodor; 105 from axilla and 11 from foot. The axilla and foot samples were divided according to genders who were 17-60 years old. (see table 1 and 2)

Table 1: The number of axilla swabs according to genders

Gender	Number of samples	Percentages (%)
Male	50	47.6
Female	55	52.4
Total	105	100%

Table 2: The number of foot swabs according to genders

Gender	Number of samples	Percentages (%)
Male	6	54.55
Female	5	45.45
Total	114	100%

2-Bacterial diagnosis

Our data showed that *S. epidermidis* was the most common isolated bacteria (from axilla 46.95% and from foot 39.13%) compared to *Corynebacterium spp* 30.49%, 34.78% and *S. aureus* 22.56%, 26.09% of the total samples from foot and axillary regions, respectively (see table 3 and 4).

Table 3: Bacterial species isolated from axilla region

Bacterial species	Number and percentages of isolation
S. epidermidis	77 (46.95%)
Corynebacteriums pp	50 (30.49%)
S. aureus	37 (22.56%)
Total	164 (100%)

Table 4: Bacterial species isolated from foot region

Bacterial species	Number and percentages of isolation
S. epidermidis	9 (39.13%)
Corynebacteriumspp	8 (34.78%)
S. aureus	6 (26.09%
Total	23 (100%)

Corynebacterium spp are Gram-positive and aerobic club-shaped bacilli. Their colonies are black-to-gray colonies, able to reduce tellurite to tellurium on Tellurite agar. *Corynebacterium* spp are catalase positive, oxidase negative. In addition, several biochemical tests were performed to confirm *Corynebacterium* identification (7,9). *Staphylococcus* is Gram-positive cocci, catalase positive, and mannitol fermented bacteria (10).

Our data was in agreement with study of (3) who found that the most predominant isolated bacteria from axilla was *Staphylococcus* spp and *Corynebacterium* spp which are often existed together and contributed to the malodor of axilla. The more bacterial numbers there, the more aromatic is produced. Our results were closed to the data of the work conducted by (11), who referred to the presence of these bacterial species as a "commensals" colonizing the skin of axilla.

Taylor et al. (2003) indicated that staphylococcal and then aerobic coryneform species are the most predominant microflora that reside at axilary region and responsible for malodor. Staphylococcal spp are responsible for biotransformation of glycerol and lactic acid to the short-chain volatile fatty acids C2 (acetic acid) and C3 (propionic acid). In addition, Staphylococci at axilary area play a role in converting leucine, a branched amino acids, to short-chain strong odorous volatile fatty acids , such as isovalerate which increase the unpleasant smell of axilla (3).

Recently, several articles have been published about medical importance of *Corynebacterium* spp as the main cause of the smell of armpit and foot (12,13). It was noticed that skin and internal organs are frequently suspected to *Corynebacterium* spp infections especially, when medical devices such as intravascular cathrters and artificial valves are utilized (12). Study of (13) mentioned that immunesuppressed and old individual are mostly exposed to *Corynebacterium spp* infections, such as pitted keratolysis (14). *Corynebacterium spp*, which cause undesirable smell of foot, was isolated from same individuals had malodor in axilla region (11) indicated that skin is a natural inhabitants of this microorganism.

3- Factors effecting antimicrobial activity of alcoholic gel

a- Shaving of axilla area

Table 5: Shaving effect on alcoholic gel activity and number of bacterial colonies per plate.

Shaving of axilla	No. of colonies before treatment	No. of colonies after 2 hr treatment	No. of colonies after 4 hr treatment
Yes	137.96	2.71	46.10
No	190.83	3.89	29.45
LSD Value	28.66*	NS	NS

A significant difference at $P \leq 0.05$ was identified in the number of bacterial colonies between people who shaved their axilla region compared to whom did not shave. After 2 hr of application alcoholic gel on axillary area, the number of bacteria of shaved skin was lower than the bacterial counts of non-shaved (see table 5).

The odor-causing bacteria grow in humid and warm places. The huge number of bacterial cell in non-shaved area is associated with the availability of nutrients such as, corticosteroids and fatty acids, which are secreted from the axillary glands and aggregated on the hair. This explanation was confirmed by (14) who referred to the relationship between the number of glands and malodor production. A larger number of endothelial glands are existed, a stronger unpleasant odor is produced. Regarding to the types of glands, the study of (15) indicated that the extracellular glands are capable of producing higher level of sweat in comparison to the endocrine glands. The crucial point of the odor formation is the long time of sweat sticking on the hair and skin of axilla which enhances bacterial growing and proliferation (16).

After application of alcoholic gel, there were no significant differences identified at $P \leq 0.05$ in the number of bacterial colonies between the shaved and non-shaved groups. The author thinks that existing of bacterial cells is related to sweat production but not presence of hair. Our findings were correlated with (17) who found no significant differences in odor production between the shaved and non-shaved groups.

b-Using of deodorants

Table 6: Effect of deodorants on the activity of alcoholic gel and the number bacterial colonies

Using of Deodorants	No. of colonies before alcoholic gel treatment	No. of colonies after 2 hr of alcoholic gel treatment	No. of colonies after 4hr of alcoholic gel treatment
Yes	123.10	3.33	14.58
NO	201.10	3.63	44.66
LSD Value	40.75*	NS	25.78*

A significant difference at $P \leq 0.05$ in number of bacterial colonies was noticed between people who used deodorants before alcoholic gel treatment and whom did not (see table 6). Our data were closed findings of (18) who evaluated antibacterial activity of deodorants. The authors concluded that all types of tested deodorants contributed in reducing the pH of the skin, and inhibiting bacterial growth at a high rate, but temporarily. Bacterial cells were re-growing and multiplying a after certain period of time based on; brand and concentration of deodorants, in addition to the level of sweating (18).

After alcoholic gel application, The colonies of isolated bacteria were counted at certain time points (2, 4, 5 and 10 hr) in order to determine the time point at which gel could show its higher activity. The best time was 2 hr after gel application, at which bacterial colonies were highly reduced. The active substances of the gel are destroying cell wall and plasma membrane of bacteria, and eventually, eliminated 99.99% of the microorganisms (19, 20). However the bacterial cells begin to reactivate and reproduce after 10 hr when gel activity is expired.

c-Effect of age

Table 7: Effect of age group and alcoholic gel activity on number of bacterial colonies

Age groups	No. of colonies before alcoholic gel treatment	No. of colonies after 2 hr alcoholic gel treatment	No. of colonies after 4 hr alcoholic gel treatment
≤20	176.19	3.33	26.74
21-30	210.77	5.57	48.80
31-40	159.25	0.00	54.83
>40	123.20	0.00	29.00
LSD Value	47.73*	NS	NS

In regard age groups, this study included people between 20-60 years old. A higher number of bacterial colonies was reported at age group 21-30 years, the adolescent age. While a lower number was reported at age >40 years old (see table 7). This results were closed to the findings of (21) who stated that the malodor of axilla changes when individuals become elderly at which the components of sweat and the activity of the glands are changed. The unpleasant herbal smell of elderly people is due to formation of N-Nomenal which is produced due to the breakdown of unsaturated fatty acids by Corynebacterium (21).

d- Effect of foot washing

Table 8: Effect of foot washing on alcoholic gel activity and number of bacterial colonies

Foot washing	No. of colonies before alcoholic gel treatment	No. of colonies after 2 hr alcoholic gel treatment	No. of colonies after 4hr alcoholic gel treatment	No. of colonies after 4hr alcoholic gel treatment
Yes	72.33	0.00	0.00	32.00
No	496.14	141.30	434.25	423.40
LSDValue	56.33*	45.61*	67.44*	71.82*

A significant difference was reported at $P \leq 0.05$ in number of bacterial colonies between individuals who wash their foot before alcoholic gel treatment and whom did not (see table 8). Washing of foot plays a key role in inhibition of bacterial cells growth and then preventing foot malodor. In our study, we found that the effect of alcoholic gel was obvious in washed-foot group compared to non-washed foot. Our finding was in agreement with (22) who referred to importance of the personal health care to avoid unpleasant odor of human body.

Before and after alcoholic gel application, the colonies of isolated bacteria were also counted at certain time points (2, 4, 5,10 hr). The number of colonies in the washed-foot group was less than that of the non-washed foot For axillary swabs, it was observed that the bacterial count was reduced after 1-2 hr and re-grow again, during the next 8 hrs (figure 1). The antimicrobial effect of alcoholic gel at foot region was less effective than at axilla (2) as a result of the prolonged use of shoes with the socks, leading to increase sweating and reduce the effect of gel (2).

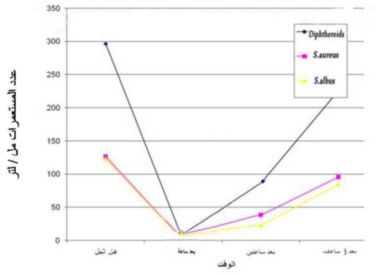


Figure 1: The number of isolated bacteria before and after using Alcoholic gel

e- Job and home residency

This work included collection of foot and axilla swabs from individuals who have different jobs, living at urban and rural areas. *Corynebacterium* spp was isolated from all above mentioned groups with no significant differences at $P \leq 0.05$ in bacterial counts among them. While a significant difference was identified in bacterial colony numbers isolated from urban and rural groups (table 9).

Data of this study confirmed that there is no one specific reason for malodor production rather than many factors participate to do so. These factors could be genetic, personal health care, hormonal or pathological status, taking specific medicine or even psychological condition (23). However, bacterial growth and malodor production depend on sweat quantity and compositions; fatty acids and steroids.

Table 9: Effect of the gender, the residence and the job on alcoholic gel activity on bacterial cells count

Gender	No. of colonies before alcoholic gel treatment	No. of colonies after 2 hr alcoholic gel treatment	No. of colonies after 4 hr alcoholic gel treatment
Male	170.80	2.92	32.07
Female	182.73	19.00	34.89
LSD Value	NS	NS	NS
Residence			
Urban	161.31	3.12	50.13
Rural	202.38	4.33	14.84
LSD Value	38.54*	NS	22.80 NS
Job			
Student	174.10	3.57	19.33
Employed	201.20	3.90	55.95
Hospital cleaner	177.60	0.00	18.14
Housewife	116.00	0.00	76.50
LSD Value	41.47*	NS	29.76*

Secretion of sweat could be hereditary type in origin. It has been found that ABCC11 gene, which is vertically transferred from parents to offspring, is responsible for the type and composition of sweat secretions (24). Furthermore, bacterial growth and hormonal secretions play an important role in changing composition of sweat, especially in women (at menopause or teenage age). It has been reported that bacterial growth was faster in teenagers compared to adults because of their hormonal secretions in addition to the stress effort by teenage individuals. In a study conducted by (25), the authors performed a psychiatric test , Trier Social Stress Test (TSST), by putting 20 of adolescents under stress coditions. The study concluded that psychological stress is increasing sweat production and the smell is stronger in males than females.

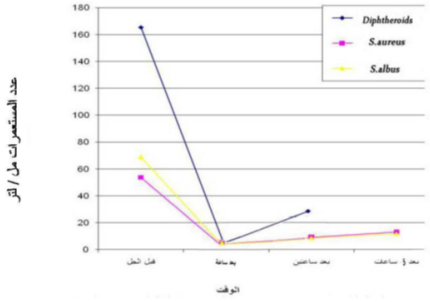
f- Bacterial species

The antibacterial activity of alcoholic gel on bacterial counts was evaluated. We found that gel has a strong inhibitory action against *Corynebacterium* spp. All of bacterial cells were inhibited after 2 hr of gel application (table 10). While S. *epidermidis and S. aureus* were capable to grow and multiply after 2hr of gel application. Staphylococcal resistance to gel is due their possession of several virulence factors enhancing antimicrobial resistance (26).

Table 10: Inhibitory effect of alcoholic gel on isolated bacteria and their colonies numbers before and after gel application (axillary swabs)

Bacterial Spp.	No. of colonies before alcoholic gel treatment	No. of colonies after 2 hr alcoholic gel treatment	No. of colonies after 4 hr alcoholic gel treatment
Corynebacterium spp	21.00±6.00	0.00±0.00A	0.00±0.00A
S. epidermidis	81.25±48.93	0.00±0.00A	13.00 ±7.34 A
S.aureus	87.25±53.09	3.62 ±2.52A	11.75 ±7.72 A
LSD Value	210.30 NS	7.737NS	44.043NS

Figure 2: Microbial growth curve of bacteria isolated from axilla. After 2 hr, the bacterial counts were significantly reduced. Re-growth and multiplication of bacterial cells occurred again because of losing antimicrobial activity after 2 hr.



4-Determination of minimum inhibitory concentration (MIC) of alcoholic gel

In our study, we performed well diffusion test in order to evaluate antimicrobial activity of alcoholic gel against *S. epidermidis* and *Corynebacterium* spp. Our data showed that 5% was the MIC that prevented bacterial growth. Using of alcoholic gel as alternative treatment was mentioned in several studies such as (27) who suggested using of plant extract, usinic acid, which had a bactericidal effect on *Corynebacterium* spp. without irritating the skin tissue. Moreover, (28) reported that extract of castor, zincricinoleate, had antimicrobial effect against *Corynebacterium Xerosis* , the causative agent of unpleasant odor of body.

In addition to being a disincentive, alcoholic gel is a skin protective and moisturizing agent. It has been used as a hand sanitizer at home or hospitals and used in pharmaceutical and personal healthcare industries. In addition, it does not damage clothing because of its quick evaporation without leaving white or yellow spots on clothing (29, 20). In comparison to gel, deodorants have several disadvantages including; (i) leaving white or yellow spots in the clothes, (ii) poisoning with its components such as, aluminum,(ii) participating in dangerous diseases such as Alzheimer's disease and breast cancer (30) (iii) their use is prohibited for individuals who had renal dysfunction (31).

REFERENCES

1. Holzle, E. (2002): Pathophysiology of sweating. Curr. Probl. Dermatol., 30: 10–22.
2. Kanlayavattanakul , M. and Lourith, N . (2011): Body malodours and their topical treatment agents, Int. J. Cosmet. Sci., 33 (4): 298–311.
3. Taylor, D.; Daulby, A.; Grimshaw, S.; James, G.; Mercer, J. and Vaziri, S. (2003): Characterization of the microflora of the human axilla, Int. J. Cosmet. Sci., 25(3): 137–145.
4. Darbre, P.D. (2005): "Aluminium, antiperspirants and breast cancer". J. Inorg. Biochem., 99 (9): 1912–1919.
5. Lankoff, A.; Banasik, A. and Duma, A. (2006): "A comet assay study reveals that aluminium induces DNA damage and inhibits the repair of radiation-induced lesions in human peripheral blood lymphocytes". Toxicol. Lett., 161 (1): 27–36.
6. Baron, E.J. and Finegold, S.M.(1990): Baily Scotts diagnostic microboilogy . 8th ed .the C.V. mosby CO., ST. louis, Baltimore, Philadelphia .
7. Holt, J. G.; Kreig, N.R.; Sneath, P.H.A.; Staley, J.T . and Williams, S.T. (1994): Bergeys Manual of Determinative Bacteriology, 9th ed. Williams and Wilkins Baltimore .,91:195-532.
8. Cruikshank, R.; Duguid, J.P.; Marmion, B.P. and Swain, R.H.A .(1975): Medical Microbiolog,vol.2 (12thed) .Churchill Livingstone (publ.) London.
9. Collee, G.J.; Faser, G .A.; Marmion, B. and Simmons, A. (1996): Laboratory starting in the diagnosis of infectivessyndrome.In :Practical medical microbiology . 14th ed

.Churchill Livingstone .UK.
10. Gillespie, S.H. and Hawkey , P.M . (2006) :Principles and Practice of Clinical Bacteriology . second Edition . 7(10) 73-98.
11. Nava, D. and Wertz, P.W. (2011): Innate immune system of skin and oral mucosa . Wiley .com : 336- 345 .
12. Moazzez, A.; Kelso, RL.; Towfigh, S.; Sohn, H. and Berne, TV. and Mason, RJ. (2007): Breast abscess bacteriologic features in the era of community-acquired methicillin-resistant Staphylococcus aureus epidemics. Arch. Surg., 142(9): 881-884.
13. Gomes, D. L.; Martins, C. A.; Faria, L. M.; Santos, L. S.; Santos, C. S.; Sabbadini, P. S.; Souza, M. C.; Alves, G. B.; Rosa, A. C.; Nagao, P. E.; Pereira, G. A.; Hirata, R. Jr. and Mattos- Guaraldi, A. L. (2009). Corynebacterium diphtheriae as an emerging pathogen in nephrostomy catheter-related infection: evaluation of traits associated with bacterial virulence. J. Med. Microbiol., 58 (11): 1419-1427.
14. Guillet, G.; Zampetti, A. and Aballain-Colloc, M.L. (2000): Correlation between bacterial population and axillary and plantar bromidrosis: study of 30 patients., Eur. J. Dermatol.; 10(1): 41-42.
15. Bovell, D.L.; Corbett, A.D.; Holmes, S.; Macdonald, A. and Marker, M. (2007): The absence of apocrine glands in the human axilla has disease pathogentic implication s , including axillary hyperhidrosis . Brirth J. Dermatol., 156(6) :1278-1286.
16. Havlíček, J.; Lenochová, P.; Oberzaucher, E.; Grammer, K. and Craig Roberts, S. (2011) : Does Length of Sampling Affect Quality of Body Odor Samples, Chem. Percept., 4:186–194.
17. Dagmar, K.; Anna, R. and Jan, H. (2012): Shaving of axillary hair has only a transient effect on perceived body odor pleasantness . Behavior. Ecol and Sociobiol., 66(4) : 569-581.
18. Stenzaly-Achtert, S.; Schölermann, A.; Schreiber, J.; Diec, K.H.; Rippke, F. and Bielfeldt, S. (2000): Axillary pH and influence of deodorants. Skin Res. Technol., 6(2):87-91.
19. Kampf, G.; Rudolf, M.; Labadie, L-C. and Barrett, S.P. (2002):Spectrum of antimicrobial activity and user acceptability of the hand disinfectant agent Sterillium Gel. J. Hospital Infect. 52: 141-147.
20. Barbut, F.; Djamdjian, L.; Neyme, D. and Passot,C.J.P. (2007): Efficacy of 2 Alcohol-Based Gels and 1 Alcohol-Based Rinse for Surgical Hand Disinfection. Infec. Cont. Hospital epidemiol., 28:1013-1015.
21. Yamazaki, S.; Hoshino, K. and kusuhara, M. (2010) : Odor associated with aging . Anti – Aging Med., 7(6): 60-65.
22. Johan, N.L. and Mats, J. O. (2010): Functional Neuronal Processing of Human Body Odors, Vitam. Horm., 83:1-23.
23. Weisfeld, G.E.; Czilli ,T.; Phillips, K.A.; Gall, J.A. and Lichtman, C.M. (2003): Possible olfaction-based mechanisms in human skin recognition and inbreeding avoidance. J. Experiment. Child Psychol. 85(3): 279-295.
24. Inoue, Y.Â.; Mori, T.Â.; Toyoda, Y.Â.; Sakurai, A.Â .; Ishikawa ,T.Â.; Mitani, Y.Â.; Hayashizaki, Y.Â.; Yoshimura,Y.Â.; Kurahashi, H.Â. and Sakai, Y.Â. (2010): Correlation of axillary osmidrosis to a SNP in the ABCC11 gene determined by the Smart Amplification Process (SmartAmp) method. J. Plastic Reconstruct. Aesthet. Surg., 63(8): 1369-1374.
25. Martin, A.; Saathoff, M. and Kuhn, F. (2010): A functional ABCC11 allele is essential in the biochemical formation of human axillary odor. J. Invest. Dermatol., 130: 529–540.
26. Greenwood, D.; Slack, R. and Peutther, J. (2002): A Guide to microbial infection, 16th ed. Elsever Science Limited. London , New York.
27. Cocchietto, M.; Skert, N.; Nimis, P.L. and Sava, G. A. (2002): Review on usnic acid, an interesting natural compound. Naturwissenschaften, 89(4):137-146.
28. Dumas, E.R.; Michaud, A.E.; Bergeron, C.; Lafrance, JL.; Mortillo, S. and Gafner, S. (2009): Deodorant effects of a supercritical hops extract: antibacterial activity against Corynebacterium xerosis and Staphylococcus epidermidis and efficacy testing of a hops/zinc ricinoleate stick in humans through the sensory evaluation of axillary deodorancy. J. Cosmet. Dermatol. 8(3):197-204.
29. Mcgrath, K.G. (2003): An earlier age of breast cancer diagnosis related to more frequent use of antiperspirants/deodorants and underarm shaving, Eur. J. Cancer Prevent., 12(6): 479-485.
30. Matthew, J.Z. and Jessica, M. (2008): Antiperspirant and Deodorant llergy. J. Clin. Aesthet. Dermatol.; 1(3):38–43.
31. Kampf, G. and Kramer, A (2004): Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs. Clin. Microbiol. Rev., 17:863-893.