Original Article

Effectiveness of topical green tea against multidrug-resistant *Staphylococcus aureus* in cases of primary pyoderma: An open controlled trial

Nagaraju Umashankar, Belliappa Pemmanda, P. Gopkumar[1], A. J. Hemalatha[2], Priya K. Sundar, H. V. Prashanth[3]

Department of Dermatology, Rajarajeswari Medical College and Hospital, Bengaluru, [1]Department of Quality Assurance, GRY Institute of Pharmacy, Khargone, [2]Department of Preventive Medicine, Oxford Institute of Medical Sciences and Research Centre, Bengaluru, [3]Department of Microbiology, Sri Siddhartha Medical College, Tumkur, Karnataka, India

Abstract

Background: Antimicrobial activity of green tea against *Staphylococcus aureus* both *in vitro* and *in vivo* has been reported recently. Studies on clinical efficacy and safety of green tea as antibacterial agent against *S. aureus* in human cases are rare.

Objectives: To evaluate the clinical effectiveness and safety of topical green tea on primary pyoderma caused by *S. aureus*. We also attempted to determine the minimum inhibitory concentration of green tea against *S. aureus* and methicillin-resistant *S. aureus*.

Methods: Open label, prospective, placebo-controlled study included community-acquired primary pyoderma cases caused by *S. aureus*. Severity grading was done on a scale of 1–5. Green tea ointment 3% and placebo ointment were used. Cure was defined on the basis of negative culture and assessment of clinical improvement. Minimum inhibitory concentration was determined by agar dilution method. Data were analyzed using Statistical Package for Social Sciences (SPSS) software version 16.

Results: Of the 372 patients, 250 received green tea and 122 received placebo. Multidrug-resistant *S. aureus* was isolated in 89.1% in green tea group and 81.1% in placebo group, respectively. Methicillin-resistant *S. aureus* was isolated in 24 patients. Cure was seen in 86% in green tea group and 6.6% in placebo group which was statistically very significant. The number of days for comprehensive cure in green tea group was 9.2 ± 6.4 days. All patients with methicillin-resistant *S. aureus* infection in the green tea group were cured. Minimum inhibitory concentration of green tea against *S. aureus* was 0.0265 ± 0.008 µg/ml and against methicillin-resistant *S. aureus* was 0.0205 ± 0.003 µg/ml.

Limitations of the Study: Comparative trial was not conducted in the same patient with different lesions; children less than seven years were not considered as the school authorities did not permit for younger children to be included in the study and true randomization and blinding of investigators were not done.

Conclusions: Green tea has a significant antibacterial effect against multidrug-resistant *S. aureus*. Minimum inhibitory concentration of green tea is established and is promising in methicillin-resistant *S. aureus* infections.

Key words: Antimicrobial activity, green tea, *Staphylococcus aureus*

Correspondence:
Dr. Nagaraju Umashankar,
Department of Dermatology,
Rajarajeswari Medical College and Hospital, Mysore Road,
Bengaluru - 560 074,
Karnataka, India.
E-mail: usdermavision@gmail.com

Access this article online	
Quick Response Code:	Website: www.ijdvl.com
	DOI: 10.4103/ijdvl.IJDVL_207_16

How to cite this article: Umashankar N, Pemmanda B, Gopkumar P, Hemalatha AJ, Sundar PK, Prashanth HV. Effectiveness of topical green tea against multidrug-resistant *Staphylococcus aureus* in cases of primary pyoderma: An open controlled trial. Indian J Dermatol Venereol Leprol 2018;84:163-8.

Received: July, 2016. **Accepted:** September, 2016.

Introduction

The development and rapid escalation of multidrug-resistant *Staphylococcus aureus* worldwide is worrisome. This is compounded by the emergence of methicillin-resistant *S. aureus* in both hospital- and community-acquired infections along with its increased rate of nasal colonization. *S. aureus* is a versatile pathogen capable of causing a variety of human infections. The capacity of *S. aureus* to colonize, produce several virulence factors, acquire drug resistance and spread has contributed to its pathogenicity.[1] It is believed that poor patient compliance and misuse or abuse of antibiotic play important roles in resistance development.[2] New paradigms for the treatment of bacterial infections are badly needed as the utility of conventional antibiotics is being eroded by the spread of multiple drug resistance. In view of the rapid spread of multidrug-resistant *S. aureus* worldwide, search for a newer antimicrobial agent which is effective and prevents the development of resistant strains of *S. aureus* is essential. Catechin-mediated effects may serve as useful indicators for the development of novel modalities for the treatment of infection.[3]

Recent data suggest that green tea contains polyphenols predominantly catechins (flavo-3-ols) which has antimicrobial activity against wide range of pathogenic bacteria including methicillin-resistant *S. aureus* both *in vitro* and *in vivo*.[4-6] Its antimicrobial effects are reported to be the result of the disruption of bacterial cell membranes by triphenols. Our study is based on this hypothesis, as effectiveness of green tea against *S. aureus* as antibacterial agent has not been studied extensively in human cases. This study was conducted to evaluate the effectiveness and safety of topical green tea on primary pyoderma caused by *S. aureus* in human cases. We also determined the minimum inhibitory concentration of green tea against *S. aureus* and methicillin-resistant *S. aureus*.

Methods

This was an open-labeled, prospective, placebo-controlled study for a period of 1 year from August 2014 to July 2015.

Study group

Primary pyoderma cases caused by *S. aureus* from schools near Rajarajeswari Medical College and Hospital, Bengaluru, India, were inducted. Inclusion criteria included positive bacterial culture and history of being untreated during the past 30 days. Clinical severity grading was done on a scale of 1–5 according to the presence of erythema, vesiculopustular eruption, scaling and crusting, discharge and edema due to inflammation. Patients having all five elements on day 1 were labeled as having score 5. Only patients with score 4 and 5 were included in the study. Patients with hypersensitivity to green tea were excluded from the study. Complete hemogram and urine examinations were done before initiating treatment. Written informed consent was obtained before study enrollment.

Green tea extract

Green tea prepared in ointment base at a concentration of 3% was used. Green tea was extracted by continuous hot percolation method.[7] One hundred grams of shade dried powdered material was packed in the Soxhlet apparatus. Active constituents were extracted using absolute alcohol at low heat. Extract was concentrated by evaporating the solvent at low temperature and semisolid extract was concentrated further by drying it over sodium sulfite in a desiccator. Final extract was dark in color with semisolid consistency with the yield of 21% w/w. Three grams of the extract was incorporated in 100 g of simple ointment and used for clinical evaluation and antimicrobial activity.

Constituents of green tea ointment (3%)

- Green tea extract: 3 g
- Wool fat: 5 g
- Hard paraffin: 5 g
- Polyethylene glycol 400 USP: 5 g
- Yellow soft paraffin: 82 g (for 100 g of ointment).

Sample size estimation

The sample size was calculated using the formula:

$$ss = \frac{z^2 \times p\,(1-p)}{c^2}$$

Where $z = 1.96$, P = Prevalence of the primary pyoderma is 35%,[8] $c = 10\%$ of prevalence.

Hence, at confidence level of 95%, calculated sample size was 373.

Study design

The research protocol was approved by the institutional ethics committee. Randomization was done on a ratio of 2:1 (green tea vs. placebo). This ratio was approved by statistician based on the literature.[9] The cross-sectional study was done for school children between 8 and 16 years. The primary pyoderma cases were identified and grouped into interventional groups, namely, green tea and placebo group. Each interventional group was chosen from random method based on which after for every two cases of green tea group and third case was taken for placebo group. Green tea ointment 3% and yellow soft paraffin as placebo were given to the patients in the respective groups and were instructed to apply to the affected sites twice daily for 15 days. The study was not blinded. No other topical or systemic antimicrobials were allowed during the study.

Assessment

The patients were evaluated initially and every 3rd day. Serial pus swabs were collected from pyoderma lesions and subjected to Gram's staining and culture. The bacterial isolates were identified using standard procedure.[10] *S. aureus* was identified on the basis of colony morphology, Gram's stain, catalase test, slide and tube coagulase test and modified Hugh Leifson's oxidation fermentation test.[11] Antibiotic sensitivity was performed on all *S. aureus* isolates using Kirby-Bauer disc diffusion method.[12] *S. aureus* ATCC 25923 was used as control. Cefoxitin was used for initial screening for methicillin resistance.[13] Isolates found resistant was further confirmed by oxacillin agar. The minimum inhibitory concentration of oxacillin was determined using agar dilution.[14] *S. aureus* ATCC 29213 was used as control. Cure was defined on the basis of negative culture and clinical improvement. Patients with score 0–1 was considered as cured, 2–3 as improved and 4–5 as treatment failure.

Minimum inhibitory concentration of green tea against *S. aureus* and methicillin-resistant *S. aureus* was determined using agar dilution method recommended by Clinical and Laboratory Standards Institute.[13] Minimum inhibitory concentration was defined as the lowest concentration that inhibited growth completely. Minimum inhibitory concentrations were recorded after 24 h of incubation at 35°C.

Data were analyzed by mean, standard deviation and Chi-square tests at 95% confidence interval from Statistical Package for Social

Sciences, International Business Machines Corporation, Armonk, New York, United States, version 16 and Microsoft Excel software.

Results

Out of 395 patients observed, a total of 380 patients of primary pyoderma caused by *S. aureus* were included in the study. Of the 372 patients who completed the study, 250 received green tea and 122 received placebo. Mean age and mean duration of lesions were almost similar in both groups [Table 1]. The age group varied from 8 to 16 years. Sex distribution in both groups was almost similar, majority being men [Table 1]. Statistically, there was no significant difference between the age, duration and sex distribution of the groups.

Impetigo contagiosa was the most common form of pyoderma in both groups [Table 2], followed by ecthyma and folliculitis. Most common site was face (48.4%) followed by legs (32.6%). *S. aureus* was isolated as single organism in 236 (94.4%) patients and 110 (90.2%) patients in green tea and placebo group, respectively [Table 3].

As per the antibiogram [Table 4], highest resistance was shown to penicillin followed by erythromycin and co-trimoxazole. Multidrug-resistant *S. aureus* was isolated in 89.1% patients in green tea group and 81.1% in placebo group. Methicillin-resistant *S. aureus* was isolated in 24 (6.5%) patients (18 patients in green tea group and 6 patients in placebo group).

Efficacy assessment was done at 0, 3, 6, 9, 12 and 15 days. On an average, the number of days for comprehensive cure with green tea group was 9.2 ± 6.4 days [Table 5]. Up to 86% in green tea group and 6.6% in placebo group were cured [Table 6]. This difference was statistically very significant ($P < 0.01$). Significantly, all patients with methicillin-resistant *S. aureus* infection in green tea group were cured. Duration for comprehensive cure was 3–9 (6 ± 2.7) days. Clinical cure of few cases are shown in Figure 1a and b (case 1 of folliculitis on right forearm in 10 years old girl); and Figure 2a and b (case 2 of Impetigo contagiosa on upper lip in 12 old years girl). In contrast, none responded in the placebo group. The duration for cure was less in methicillin-resistant *S. aureus* infections compared to that of nonmethicillin-resistant *S. aureus* infections.

Minimum inhibitory concentration of green tea against *S. aureus* was 0.0265 ± 0.008 µg/ml (control *S. aureus* ATCC 25923) and against methicillin-resistant *S. aureus* was 0.0205 ± 0.003 µg/ml (control *S. aureus* ATCC 29213). Interestingly, it was lower with methicillin-resistant *S. aureus* compared to nonmethicillin-resistant *S. aureus* strains.

Itching, burning and erythema were the adverse effects and were noticed in only one patient in green tea group. It was well tolerated and accepted by all other patients.

Discussion

Emergence of multidrug-resistant *S. aureus* both in hospital and community level is a growing menace. Multidrug-resistant *S. aureus*

Table 1: Comparison of the two groups before therapy

Parameters	GT group (*n*=250)	Placebo group (*n*=122)
Mean age (years)	12.31	11.01
Mean duration of lesions (days)	6.42	5.81
Sex (%)		
Male	74.24	81.20
Female	25.76	18.80

P=0.6854. GT: Green tea

Table 2: Types of pyoderma

Type	GT group (%)	Placebo group (%)
Impetigo contagiosa	104 (41.6)	71 (58.2)
Ecthyma	55 (22)	19 (15.6)
Folliculitis	49 (19.6)	20 (16.4)
Furunculosis	24 (9.6)	9 (7.4)
Bullous impetigo	18 (7.2)	3 (2.5)
Total	250	122

GT: Green tea

Table 3: Pattern of isolates from pyoderma

Number of isolates	GT group (%)	Placebo group (%)
Single (*S. aureus*)	236 (94.4)	110 (90.16)
Mixed (*S. aureus* + β-hemolytic streptococci)	14 (5.6)	12 (9.84)
Total	250	122

S. aureus: *Staphylococcus aureus*, GT: Green tea

Table 4: Antibiotic resistance in *Staphylococcus aureus*

Antibiotics	Number (%) of bacteria resistant	
	GT group	Placebo group
Ciprofloxacin	6 (2.4)	4 (3.3)
Co-trimoxazole	102 (40.8)	57 (46.7)
Erythromycin	168 (67.2)	80 (65.6)
Gentamicin	32 (12.8)	14 (11.5)
Oxacillin (methicillin)	18 (7.2)	6 (4.9)
Penicillin	209 (83.6)	108 (88.5)
Tetracycline	28 (11.2)	15 (12.3)
Vancomycin	0	0

P=0.9396. GT: Green tea

Table 5: Assessment of efficacy (comprehensive cure)

Days	GT group, % (*n*=250)	Placebo group, % (*n*=122)
Day 0	0	0
Day 3	30.3	2.31
Day 6	49.6	4.62
Day 9	82.5	5.34
Day 12	85.93	6.13
Day 15	86	6.56

GT: Green tea

Table 6: Assessment at the end of 15 days

Outcome	GT group, %	Placebo group, %
Cured	86	6.56
Improved	8.8	9.02
Failure	5.2	84.42

P<0.001. GT: Green tea

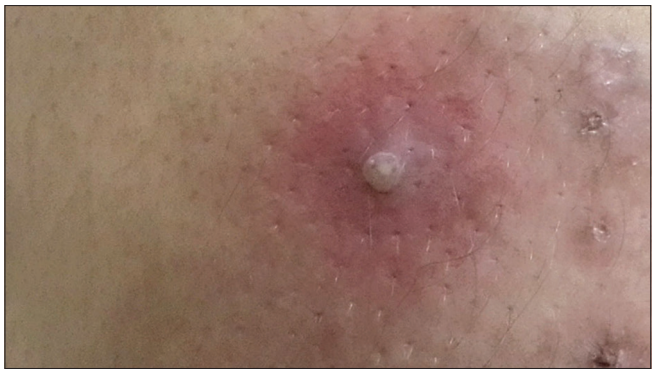

Figure 1a: Folliculitis on the right forearm in a 10-year-old girl, baseline

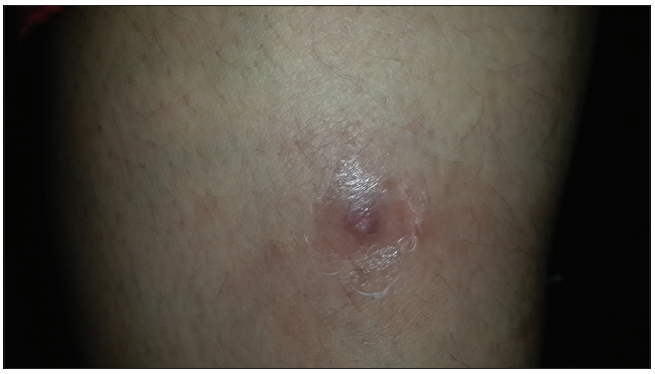

Figure 1b: Response to topical green tea, day 9

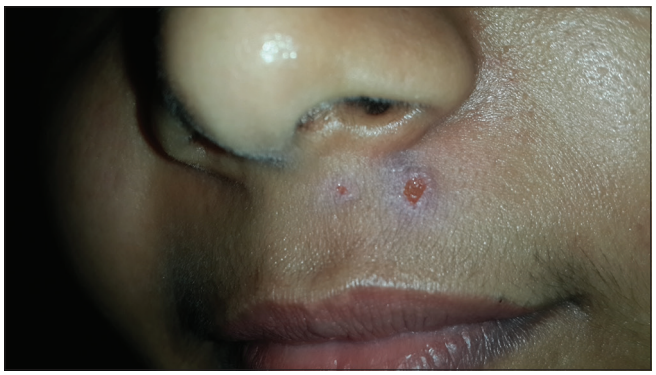

Figure 2a: Impetigo contagiosa on the upper lip in a 12-year-old girl, baseline

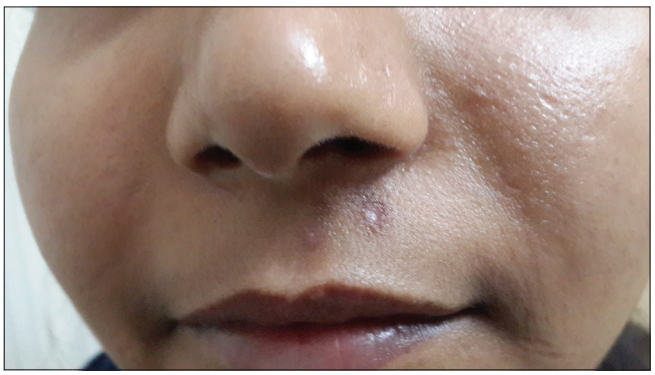

Figure 2b: Response to topical green tea, day 6

is defined as those organisms that were resistant to one or more therapeutic classes of antimicrobial agents.[15]

Emergence of multidrug-resistant bacteria is a threat to the effective treatment of serious infections, especially in hospitals.[16] The present study clearly shows that multidrug-resistant *S. aureus* has become a significant community pathogen (89.1% patients in green tea group and 81.1% in placebo group). Methicillin-resistant *S. aureus* in community-acquired pyodermas has been reported previously as 9.6% and 10.9%, whereas in the present study, it was lower (6.5%).[17,18] This very high rate of multidrug-resistant *S. aureus* in community-acquired pyodermas in the present study may be because we considered strains resistant to more than one drug as multidrug-resistant *S. aureus* based on literature.[15] Indiscriminate use and over-counter availability of different antibiotics may also be the reason for such a high resistance in this community-acquired pyoderma isolates.

According to legend, the origin of tea dates back to 2737 BC when Chinese Emperor Shen Nung enjoyed the fragrant beverage produced after dried tea leaves from a nearby bush blew into a pot of boiling water. From its discovery in ancient China 5000 years ago, tea has become the most widely consumed man-made beverage in the world.[19-22]

Tea is derived from the plant *Camellia sinensis* and is available in three main varieties: green, black and oolong tea.[23] The major chemical constituents of tea are the polyphenols which make up to 30% of the dry weight of fresh leaf. The predominant polyphenols in tea are catechins (flavon-3-ols), of which there exist four major forms: epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate.[22] Of the three types of tea, green tea has the highest catechin content, the main form being epigallocatechin-3-gallate, constituting up to 60% and has the strongest bactericidal effect.[19-22] Epigallocatechin-3-gallate and other catechins act as bactericidal agents by directly binding to peptidoglycan and interfering with the integrity and biosynthesis of cell membrane.[24,25] Yam *et al*. have used 2% green tea extract directly on clinical isolates available in their research collection.[4] However, ours being clinical study, 3% alcoholic extract in 100 g of ointment was used. When 2–4 g of ointment is used for topical application, it makes up to 0.5%–1% of green tea extract because of alcoholic extraction.

Only children studying in the third to tenth standard were considered as the school authorities did not permit for younger children to be included in the study. Hence, age group of 8–16 years was considered in our study.

The cure rate of 86% in the present study is consistent with an earlier study which showed a cure rate of 81.3% with tea ointment and 37.5% with topical tea lotion.[5] This efficacy of tea ointment is comparable to fusidic acid ointment[26] which has a cure rate of 87% and superior to soframycin ointment (framycetin sulfate and gramicidin)[5] which has a cure rate of 72%.

Other noticeable fact is methicillin-resistant *S. aureus* specifically expresses an additional penicillin binding protein termed penicillin binding protein 2a from mecA gene.[27] Epigallocatechin-3-gallate has more affinity to penicillin binding protein 2a and hence is more active against methicillin-resistant *S. aureus*. This is clearly evident in the present study with respect to duration for cure and

minimum inhibitory concentration, both of which were lower compared to nonmethicillin-resistant *S. aureus* strains. Moreover, all patients were cured. This is consistent with *in vitro* studies which reported that all methicillin-resistant *S. aureus* strains were sensitive to 2% green tea extract.[4] Earlier studies have reported minimum inhibitory concentration of 0.3 g tea/100cc water for tea lotion and 0.28 mg/ml for a crude extract of tea.[4,5] However, the present study had a minimum inhibitory concentration of 0.0205 ± 0.008 µg/ml. This difference in the minimum inhibitory concentrations may be due to the usage of different concentrations of the crude tea extract.

Other advantages of green tea as per published data include acts synergistically with other β-lactams thus reducing the minimum inhibitory concentration of β-lactams when used in combination.[28-31] Activity is stable under various physical conditions including boiling and freezing, and it acts as an antimutagenic agent thus suppressing the emergence of resistant strains.[2]

In a previous study, *S. aureus* in the anterior nares was observed in 54.4% of patients.[18] The antibiogram of *S. aureus* from pus and the anterior nares was similar in 49% of patients, suggesting that the pathogen might have originated from the colonized site. Thus, green tea may be suitable as topical agents for the treatment of these colonized sites and reduce the incidence of endogenous infections. However, clinical trials are required to confirm this.

In a study by Sharquie, no adverse effects to topical green tea were observed in children.[5] In our study, itching, burning and erythema were the adverse effects and were noticed in only one patient in green tea group.

Limitations of the study

A comparative trial of different agents for individual lesions in the same patient would have been preferred. Children less than seven years were not included in the study because of a logistic issue of permission from school authorities. True randomization and blinding of the investigators would have been ideal.

Conclusions

We were unable to find any previous clinical trial of green tea against community-acquired primary pyodermas caused by *S. aureus* in human cases. This study has clearly shown that green tea has a significant antibacterial effect against multidrug-resistant *S. aureus* and is well tolerated. Minimum inhibitory concentration of green tea is established. It could be one of the potential drugs in future for the treatment of methicillin-resistant *S. aureus* infections in cases of primary pyoderma. Other infections caused by methicillin-resistant *S. aureus* need to be evaluated with green tea extract as a possible treatment option. Further comparative clinical trials are needed involving a larger study population to support this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Archer GL. *Staphylococcus aureus*: A well-armed pathogen. Clin Infect Dis 1998;26:1179-81.
2. Pillai SP, Pillai CA, Shankel DM, Mitscher LA. The ability of certain antimutagenic agents to prevent development of antibiotic resistance. Mutat Res 2001;496:61-73.
3. Taylor PW, Stapleton PD, Paul Luzio J. New ways to treat bacterial infections. Drug Discov Today 2002;7:1086-91.
4. Yam TS, Shah S, Hamilton-Miller JM. Microbiological activity of whole and fractionated crude extracts of tea (*Camellia sinensis*), and of tea components. FEMS Microbiol Lett 1997;152:169-74.
5. Sharquie KE, al-Turfi IA, al-Salloum SM. The antibacterial activity of tea *in vitro* and *in vivo* (in patients with impetigo contagiosa). J Dermatol 2000;27:706-10.
6. Hara Y. Green Tea: Health Benefits and Applications. New York, USA: Marcel Dekker; 2001.
7. Handa SS. An overview of extraction techniques for medicinal and aromatic plants. In: Handa SS, Khanuja SP, Longo G, Rakesh DD, editors. Extraction Technologies for Medicinal and Aromatic Plants. 1st ed. Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology; 2008. p. 21-52.
8. Epidemiology and Management of Common Skin Diseases in Children in Developing Countries. Available from: http://www.who.int/maternal_child_adolescent/documents/fch_cah_05_12/en. [Last accessed on 2014 Jul 26].
9. Korn EL, Freidlin B. Outcome – Adaptive randomization: Is it useful? J Clin Oncol 2011;29:771-6.
10. Kloos WE, Bannerman TL. Staphylococcus and micrococcus. In: Murray PR, Baron JE, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of Clinical Microbiology. 6th ed. Washington, DC: American Society for Microbiology; 1995. p. 282-99.
11. Collee JG, Miks RS, Watt B. Tests for the identification of bacteria. In: Collee JG, Freser AG, Marmion BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14th ed. New York: Churchill Livingstone; 1996. p. 131-49.
12. Clinical and Laboratory Standards Institute (CLSI). Approved Standards: M02-A11-Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard. 11th ed. Wayne, PA: Clinical and Laboratory Standards Institute (CLSI); 2015.
13. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI Document M100-S24. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
14. Hindler JA, Jorgensen JH. Antimicrobial susceptibility testing. In: Mahon CR, Manuselis G, editors. Textbook of Diagnostic Microbiology. 5th ed. Philadelphia: W.B. Saunders; 2015. p. 274-314.
15. Siegel JD, Rhinehart E, Jackson M, Chiarello L; Healthcare Infection Control Practises Advisory Committee. Management of Multidrug-Resistant Organisms in Healthcare Settings; 2006. Available from: http://www.cdc.gov/ncidod/dhqp/pdf/ar/mdroGuideline2006.pdf. [Last accessed on 2016 Jul 04].
16. PHLS Report. Antimicrobial Resistance in 2000. England and Wales. London, UK: Public Health Laboratory Service; 2002.
17. Thind P, Prakash SK, Wadhwa A, Garg VK, Pati B. Bacteriological profile of community-acquired pyodermas with special reference to methicillin resistant *Staphylococcus aureus*. Indian J Dermatol Venereol Leprol 2010;76:572-4.
18. Nagaraju U, Bhat G, Kuruvila M, Pai GS, Jayalakshmi B, Babu RP. Methicillin-resistant *Staphylococcus aureus* in community-acquired pyoderma. Int J Dermatol 2004;43:412-4.
19. Balentine D. Tea and health. Crit Rev Food Sci Nutr 1997;37:691-2.
20. Balentine DA, Wiseman SA, Bouwens LC. The chemistry of tea flavonoids. Crit Rev Food Sci Nutr 1997;37:693-704.
21. Katiyar SK, Mukhtar H. Tea consumption and cancer. World Rev Nutr Diet 1996;79:154-84.
22. Mitscher LA, Jung M, Shankel D, Dou JH, Steele L, Pillai SP. Chemoprotection: A review of the potential therapeutic antioxidant properties of green tea (*Camellia sinensis*) and certain of its constituents. Med Res Rev 1997;17:327-65.
23. Alexis AF, Jones VA, Stiller MJ. Potential therapeutic applications of tea in dermatology. Int J Dermatol 1999;38:735-43.
24. Ikigai H, Nakae T, Hara Y, Shimamura T. Bactericidal catechins damage the lipid bilayer. Biochim Biophys Acta 1993;1147:132-6.

25. Taylor PW, Hamilton-Miller JM, Stapleton PD. Antimicrobial properties of green tea catechins. Food Sci Technol Bull 2005;2:71-81.
26. Gilbert M. Topical 2% mupirocin versus 2% fusidic acid ointment in the treatment of primary and secondary skin infections. J Am Acad Dermatol 1989;20:1083-7.
27. Stapleton PD, Taylor PW. Methicillin resistance in *Staphylococcus aureus*: mechanisms and modulation. Sci Prog 2002;85(Pt 1):57-72.
28. Shiota S, Shimizu M, Mizushima T, Ito H, Hatano T, Yoshida T, *et al.* Marked reduction in the minimum inhibitory concentration (MIC) of beta-lactams in methicillin-resistant *Staphylococcus aureus* produced by epicatechin gallate, an ingredient of green tea (*Camellia sinensis*). Biol Pharm Bull 1999;22:1388-90.
29. Stapleton PD, Shah S, Anderson JC, Hara Y, Hamilton-Miller JM, Taylor PW. Modulation of beta-lactam resistance in *Staphylococcus aureus* by catechins and gallates. Int J Antimicrob Agents 2004;23:462-7.
30. Zhao WH, Hu ZQ, Okubo S, Hara Y, Shimamura T. Mechanism of synergy between epigallocatechin gallate and beta-lactams against methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2001;45:1737-42.
31. Kono K, Tatara I, Takeda S, Arakawa K, Hara Y. Antibacterial activity of epigallocatechin gallate against methicillin-resistant *Staphylococcus aureus*. Kansenshogaku Zasshi 1994;68:1518-22.