TITLE PAGE

- Staphylococcus aureus and chronic folliculocentric pustuloses of the scalp – cause or association?

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- Two figures

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DEAR EDITOR, The aetiology of chronic scalp folliculitis (SF), folliculitis decalvans (FD), tufted folliculitis (TF), acne nuchae keloidalis (ANK), dissecting cellulitis (DCS) and other similar entities is not well established but these conditions share similar features including chronic scarring folliculocentric pustules localised to the scalp, response to antibiotic therapy and many show the presence of *Staphylococcus aureus* (SA). We have collectively termed these conditions chronic folliculocentric pustuloses of the scalp (CFPS), to emphasise their overlapping characteristics which suggest that there may be overlapping components in their pathogenesis, but acknowledge that individually there are distinguishing features which would imply that further contribution from distinct aetiological factors are likely to account for clinical variation in phenotype.

The suggestion that SA plays a major role in the pathogenesis of CFPS is supported by reports showing microbiological detection[1, 2] and effectiveness of anti-SA antibiotics[2, 3]. However, it is recognised that increasing rates of resistance to antibiotics are associated with injudicious prescribing and a major call for antibiotic stewardship is underway in healthcare organisations such as the National Health Service[4]. Furthermore, presence of SA is not universal from pustules of CFPS, and treatment consensus for these cases is lacking. Indeed, effectiveness of anti-SA therapy does not always correlate with the presence of SA. Therefore the precise place of anti-SA antibiotics in the management of CFPS is uncertain.

To address this equipoise, we reviewed all cases of CFPS (n=80) seen in our department from 2006-2013. We hypothesised that SA culture negative CFPS may represent a false-negative finding if SA was deep seated within a biofilm[5], and if identified, may support the use of aggressive anti-SA treatment. Thus, we employed a highly sensitive, culture independent test to detect pathogens (including SA) with 16s rDNA gene sequence analysis with broad range and SA-specific approaches in three selected SA-negative but anti-staphylococcal antibiotic responsive cases.

CFPS cases and microbiological results were identified through our hospital computer system. Whilst there are distinct limitations in a retrospective analysis, we run an electronic patient record and laboratory microbiology results system, making data collection as reliable as possible. Response to treatment was assessed from the recorded physician notes. In the three selected patients (Supplementary Table 1), all anti-microbial treatments were stopped 2 weeks prior to further investigations. Skin swabs were taken prior to pre-biopsy sterilisation. Briefly, sterile cotton buds were moistened with Probact Amies Clear medium, before swabbing a pustule with a rolling swab method and were received in the microbiology laboratory <12 hours for plating. Three punch biopsies (4mm) were taken from separate active folliculitic areas. Biopsies were sent for histology, tissue culture, and 16S rDNA sequencing.

We identified 80 patients (age range 19-78 years) with CFPS (Supplementary Table 2): 72 (90%) males and 8 (10%) females. The cohort of men were significantly younger (median 40.1 vs. 58.5 years; p=0.002). 40% of all cases comprised more severe forms of CFPS (FD/ANK/DCS). Microbiological sampling was undertaken in 60/80 (75%) cases. Although nasal swabs, would have been of value in a study of colonisation, these were not routine practice carried out in our department. SA was isolated in 25/60 (41.7%) of the cases, and was more prevalent in FD (11/21, 52.4%) as compared to SF (11/36, 30.6%). No significant association was identified between SA-status, age, gender or duration of disease.

158 treatment episodes composed of topical (n=15) and systemic (n=143) treatments were prescribed. Commonly used antibiotics are summarised (Fig. 1a&b). To compare the effectiveness of treatments with respect to SA status, we grouped the cases according to therapy regimen: tetracyclines (doxycycline/minocycline/lymecycline); rifampicin/clindamycin alone or in combination; Penicillins (flucloxacillin/amoxicillin/clavulanate); and topical antimicrobial treatment. Treatment responses were characterised from the physicians global assessment and were classified as cleared, partial response, no improvement. We found no significant difference in effectiveness between any of the anti-SA treatments (p=0.47) respective to SA status (Fig. 2a). The low number of cases of FD in this series, precluded detailed inter-regimen statistics. However, although overall response rate in FD was greater for rifampicin combinations as opposed to tetracyclines, differences in response were minimal between those with or without SA when treated with rifampicin, or those with or without SA treated with tetracyclines (Fig. 2b).

Routine histological examination in the three patients who were SA-negative yet anti-SA therapy responders (underlined in Supplementary Table 2) showed neutrophilic perifollicular inflammation. Skin swab, tissue culture, 16S rDNA PCR and SA specific real-time PCR in all three cases were negative for SA. Consistent with the normal microbiome of scalp follicles, *P. acnes* was identified in all three cases and confirmed the sensitivity of this approach.

SF, FD and TF describe a spectrum of CFPS with varying degrees of scarring. The exact aetiology is unknown but it has been suggested to be mediated by SA[1, 2]. Yet, SA was only isolated in 41.7% of our cohort. Indeed, the milder subgroup (SF) showed SA-positivity in only 30.6% of cases, which is similar to the prevalence of SA carriage in our local healthy adult population[6]. Furthermore, highly potent anti-SA regimens (rifampicin/clindamycin combination) showed similarly favourable responses in both SA-positive (77.8%) and SA-negative (66.7%) cases. This suggests the presence of SA is not critical to the effectiveness of anti-SA therapy which raises the possibility that other non-antimicrobial effects may be important.

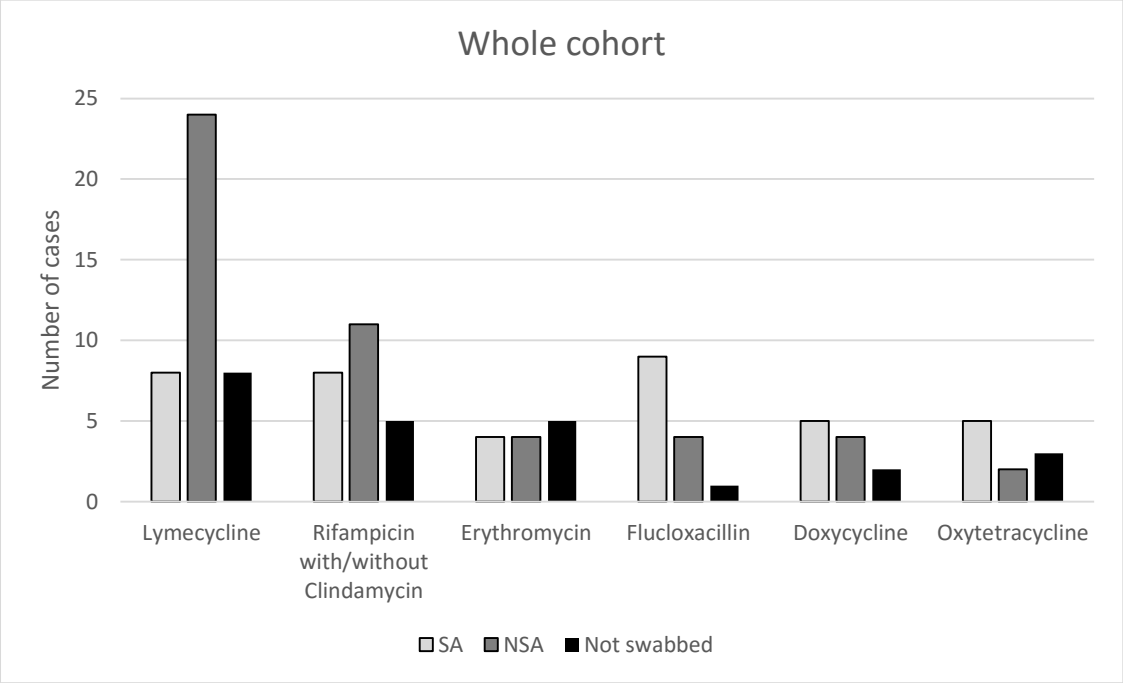
16s rDNA sequence analysis to identify pathogens in a culture independent manner has been shown to be useful where normal culture has failed, and is increasingly utilised in other clinical situations[7-9]. To our surprise, from the three selected cases whose CFPS responded to anti-SA therapy, both broad range and SA-specific (100-1000 fold more sensitive) 16S rDNA PCR did not identify SA. Thus, the identification of SA in only 31-52% of cultures strongly questions the pathogenic role of SA and supports the hypothesis that non-antimicrobial/anti-inflammatory actions of treatment may be critical to antibiotic efficacy in CFPS[1, 10, 11]. In light of the recently characterised neutrophilic dermatoses associated with mutations in the PSTPIP1 gene[12], it would be interesting to speculate whether a proportion of CFPS cases actually represent an autoinflammatory defect in the IL-1 pathway.

In summary, we suggest a collective term, chronic folliculocentric pustuloses (CFPS) of the scalp, which highlights overlapping clinicopathological features and may suggest some common aetiological factors, although we acknowledge the differences in clinical presentation of this group of disorders. Whilst anti-SA therapy is effective in many cases of CFPS, clinical response to anti-SA therapies may be effective even when SA is absent. This report provides increasing evidence to question the pathogenic role of SA, non-antimicrobial actions of anti-SA regimen and supports the possibility of immunological dysfunction as in other folliculitic conditions such as hidradenitis suppurativa[13].

References

1. Bastida J, Valeron-Almazan P, Santana-Molina N, Medina-Gil C, Carretero-Hernandez G. Treatment of folliculitis decalvans with tacrolimus ointment. Int J Dermatol. 2012 Feb;51 (2):216-20. PubMed PMID: 22250634.
2. Powell JJ, Dawber RP, Gatter K. Folliculitis decalvans including tufted folliculitis: clinical, histological and therapeutic findings. Br J Dermatol. 1999 Feb;140 (2):328-33. PubMed PMID: 10233232.
3. Sillani C, Bin Z, Ying Z, Zeming C, Jian Y, Xingqi Z. Effective treatment of folliculitis decalvans using selected antimicrobial agents. International journal of trichology. 2010;2 (1):20.
4. Dixon J, Duncan CJ. Importance of antimicrobial stewardship to the English National Health Service. Infect Drug Resist. 2014;7:145-52. PubMed PMID: 24936131. Pubmed Central PMCID: 4047980.
5. Matard B, Meylheuc T, Briandet R, Casin I, Assouly P, Cavelier-balloy B, et al. First evidence of bacterial biofilms in the anaerobe part of scalp hair follicles: a pilot comparative study in folliculitis decalvans. J Eur Acad Dermatol Venereol. 2013 Jul;27 (7):853-60. PubMed PMID: 22779760.
6. Gamblin J, Jefferies JM, Harris S, Ahmad N, Marsh P, Faust SN, et al. Nasal self-swabbing for estimating the prevalence of Staphylococcus aureus in the community. J Med Microbiol. 2013 Mar;62 (Pt 3):437-40. PubMed PMID: 23222858. Epub 2012/12/12. eng.
7. Rudkjobing VB, Thomsen TR, Alhede M, Kragh KN, Nielsen PH, Johansen UR, et al. True microbiota involved in chronic lung infection of cystic fibrosis patients found by culturing and 16S rRNA gene analysis. J Clin Microbiol. 2011 Dec;49 (12):4352-5. PubMed PMID: 22012018. Pubmed Central PMCID: 3233003.
8. Welinder-Olsson C, Dotevall L, Hogevik H, Jungnelius R, Trollfors B, Wahl M, et al. Comparison of broad-range bacterial PCR and culture of cerebrospinal fluid for diagnosis of community-acquired bacterial meningitis. Clin Microbiol Infect. 2007 Sep;13 (9):879-86. PubMed PMID: 17608746.
9. Drancourt M, Berger P, Terrada C, Bodaghi B, Conrath J, Raoult D, et al. High prevalence of fastidious bacteria in 1520 cases of uveitis of unknown etiology. Medicine (Baltimore). 2008 May;87 (3):167-76. PubMed PMID: 18520326.
10. Mihaljevic N, von den Driesch P. Successful use of infliximab in a patient with recalcitrant folliculitis decalvans. J Dtsch Dermatol Ges. 2012 Aug;10 (8):589-90. PubMed PMID: 22676386.
11. Labro MT. Immunomodulatory effects of antimicrobial agents. Part I: antibacterial and antiviral agents. Expert Rev Anti Infect Ther. 2012 Mar;10 (3):319-40. PubMed PMID: 22397566. Epub 2012/03/09. eng.
12. Ferguson PJ, Lokuta MA, El-Shanti HI, Muhle L, Bing X, Huttenlocher A. Neutrophil dysfunction in a family with a SAPHO syndrome-like phenotype. Arthritis Rheum. 2008 Oct;58 (10):3264-9. PubMed PMID: 18821685.
13. Jemec GB. Hidradenitis suppurativa and immune dysregulation. Br J Dermatol. 2012 Feb;166 (2):237-8. PubMed PMID: 22268854. Epub 2012/01/25. eng.

(a)



(b)

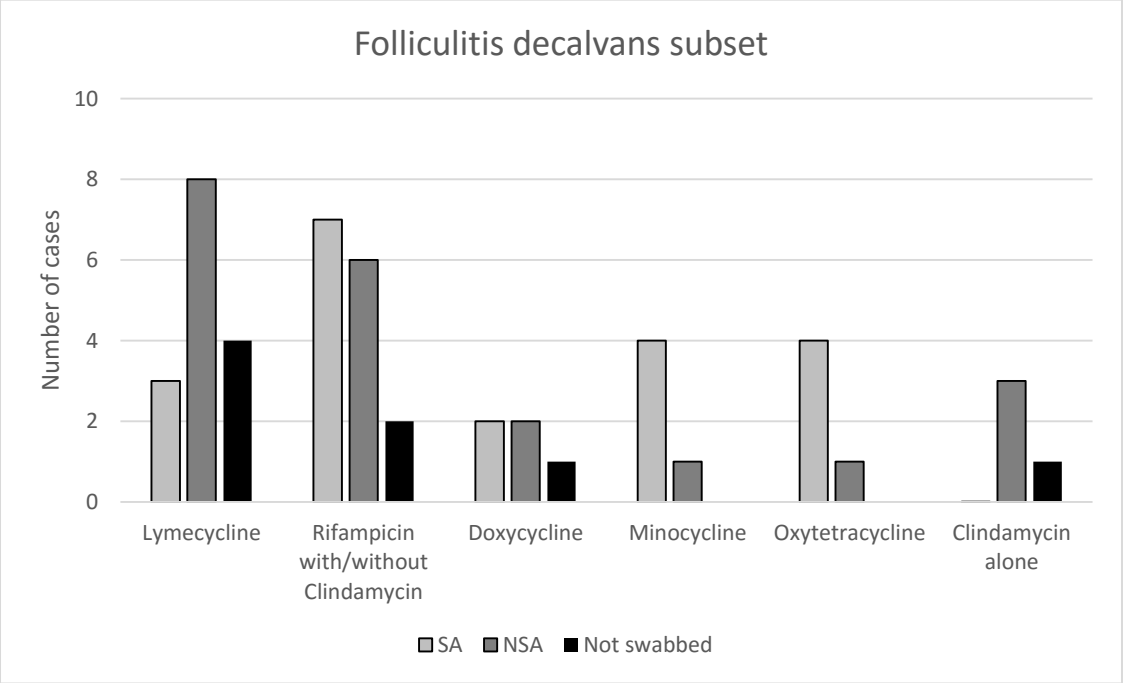
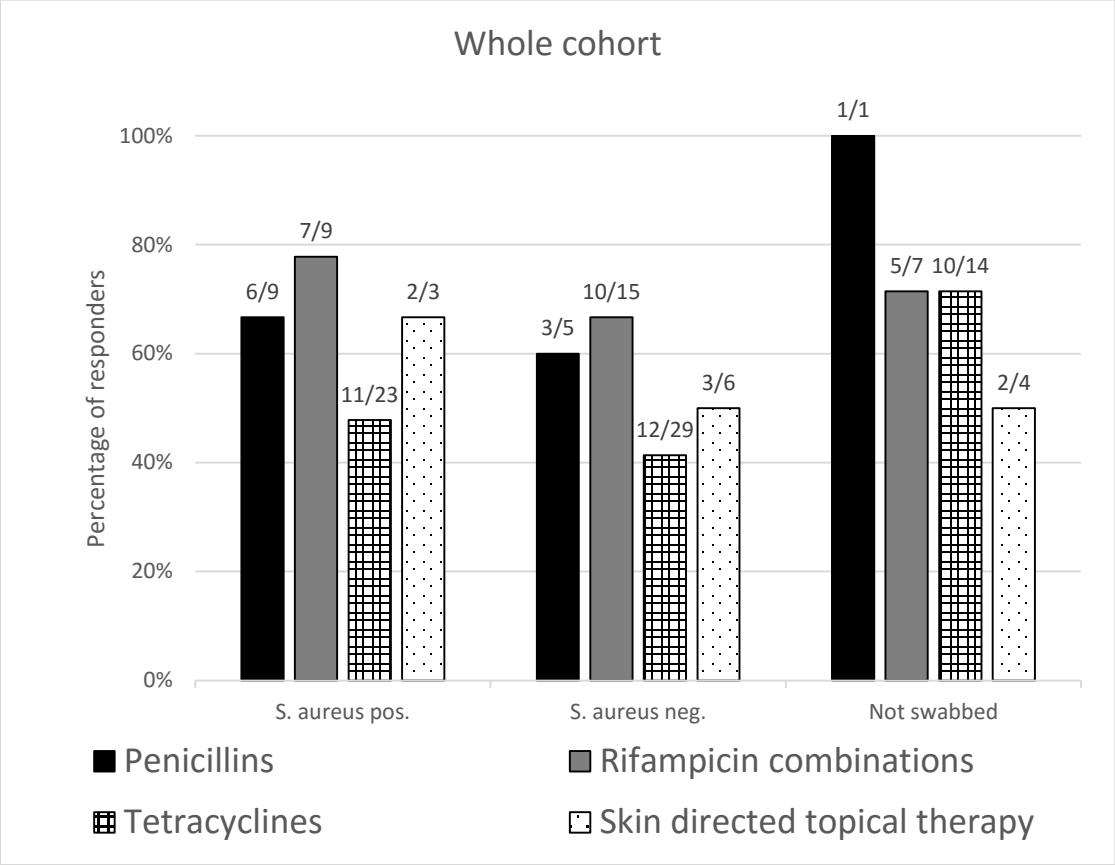


Figure 1. Antibiotics used to treat CFPS. (a) Whole cohort. (b) Folliculitis decalvans subset.

Staphylococcus aureus positive (SA; Yellow bars); Staphylococcus aureus negative (NSA; red bars); Not swabbed (purple bar).

(a)



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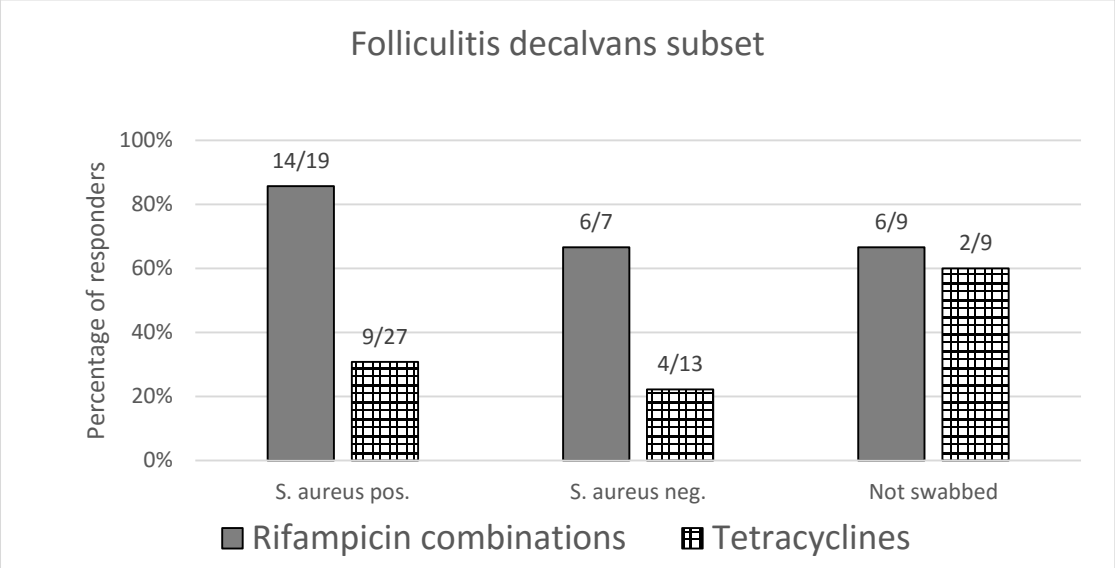

Figure 2. Response rate (including partial responders) of most commonly used antibiotic groups. (a) Whole cohort. (b) Folliculitis decalvans subset. Responding number of cases / total number of cases per group noted above columns. Low numbers for penicillin and skin directed treatment not shown.

Penicillins (flucloxacillin, amoxicillin/clavulanate); Rifampicin combinations (alone or in combination); Tetracyclines (doxycycline, minocycline or lymecycline). Skin directed topical therapy (topical antibiotics, antiseptics and anti-inflammatories)

Patient	1	2	3
Age	32	56	51
Sex	m	m	m
Condition	SF	SF	SF
Duration	6 years	20 years	14 years
Skin co-morbidities	atopic eczema	atopic eczema	nil
Major medical co-morbidities	nil	nil	nil
Swabs	negative	negative	negative
Previous partial response	Lymecycline	Lymecycline	Amoxicillin/clavulanic acid
Rifampicin combination Rx	highly effective	highly effective	highly effective
Effect of withdrawing R/C	recurrence	recurrence	recurrence

Supplementary Table 1. Characteristics of three selected patients.

M: male, SF: Scalp folliculitis

No.	Age	Sex	Type of folliculitis	Duration of problem (months)	*Staphylococcus Aureus* positivity	Treatments received
1	58	F	FD	7	Negative	L, R+C
2	24	M	FD	29	ni	Nizoral
3	36	M	FD	48	Positive	L, R+F, R+C
4	31	M	FD	120	Negative	E, L+D, I
5	52	M	FD	ni	Positive	L, M
6	45	M	FD	84	Positive	O, R+C
7	70	F	FD	ni	Positive	M, protopic
8	53	M	FD	108	Negative	L, C+T
9	56	M	FD	360	Negative	D, L, R+C, C, R+L, S
10	40	M	FD	ni	Positive	I, R+C, M, S
11	38	M	FD	12	Negative	L, R+C
12	32	M	FD	72	ni	L
13	32	M	FD	60	ni	L, L+BP
14	36	M	Scalp folliculitis	60	Negative	L, S, I
15	32	M	FD	5	Negative	M
16	24	M	FD	ni	Positive	O, R+C
17	35	M	FD	36	Negative	L
18	52	M	FD	ni	Positive	R+C, O
19	36	M	Scalp folliculitis	ni	Negative	R+C
20	26	M	FD	48	Negative	O
21	32	M	FD	48	Negative	Topical clindamycin
22	46	M	FD	144	Positive	R+D
23	38	M	FD	ni	Negative	F, R+C
24	29	M	FD	72	Positive	D+DalacinT
25	64	F	FD	48	ni	D+T, E
26	59	F	FD	21	Positive	O
27	38	M	FD	108	Positive	Amoxicillin, M, D
28	42	M	FD	84	ni	R+C, L, C
29	58	M	FD	ni	ni	R+C
30	51	M	Scalp folliculitis	156	Negative	R+C, R+Cl, Coamoxiclav, acitretin+Coamoxiclav, C
31	28	M	scalp folliculitis	60	ni	O, M, Cl+T
32	39	M	Scalp folliculitis	120	Negative	L, D, Ketoconazole
33	46	M	Scalp folliculitis	120	ni	M, I
34	33	M	Scalp folliculitis	ni	Negative	I
35	66	M	Scalp folliculitis	ni	Negative	L, M, F+protopic
36	41	M	Scalp folliculitis	ni	Negative	I
37	34	M	Scalp folliculitis	6	Negative	L
38	26	M	Scalp folliculitis	60	Positive	L
39	48	M	Scalp folliculitis	ni	Negative	Zineryt & betadine shampoo
40	40	M	Scalp folliculitis	24	Negative	L
41	54	F	Scalp folliculitis	ni	ni	L+topical steroid
42	52	M	scalp folliculitis	ni	ni	R, S, E, C+T
43	32	M	scalp folliculitis	84	Negative	R+C, L+DalacinT
44	24	M	scalp folliculitis	24	Negative	E, L, R+C
45	45	M	Scalp folliculitis	ni	ni	L
46	71	M	Scalp folliculitis	ni	Negative	L
47	78	F	scalp folliculitis	24	ni	E, topical steroid
48	75	F	scalp folliculitis	ni	ni	bettamouse
49	65	M	scalp folliculitis	ni	Positive	F, L
50	21	M	scalp folliculitis	ni	Negative	L
51	41	M	Dissecting scalp folliculitis	120	ni	R+D, O, R+C
52	46	M	scalp folliculitis	144	Positive	D500
53	26	M	Acne nuchae keloidalis	24	Positive	L, F, R+C, I, zindaclin
54	35	M	scalp folliculitis	3	Positive	F
55	50	M	scalp folliculitis	ni	Negative	chlorhexidine
56	53	M	scalp folliculitis	ni	Positive	D

No.	Age	Sex	Type of folliculitis	Duration of problem (months)	*Staphylococcus Aureus* positivity	Treatments received
57	43	M	scalp folliculitis	ni	ni	D500
58	32	M	scalp folliculitis	72	Positive	D, O, R+C
59	19	M	scalp folliculitis	18	Negative	E, L, T, D
60	36	M	scalp folliculitis	18	Negative	L
61	50	M	scalp folliculitis	12	ni	L, O+dalcinT
62	51	M	scalp folliculitis	12	Negative	D500
63	25	M	scalp folliculitis	ni	Positive	L
64	42	M	Acne nuchae keloidalis	48	Positive	F, T, M, L, E
65	40	M	scalp folliculitis	ni	Negative	F + fucidin
66	30	M	scalp folliculitis	ni	ni	F, E
67	68	M	scalp folliculitis	60	Negative	O, L, zindaclin
68	19	M	scalp folliculitis	ni	Negative	L
69	41	M	scalp folliculitis	ni	ni	L
70	31	M	scalp folliculitis	ni	Negative	L
71	46	M	scalp folliculitis	ni	Positive	F
72	39	M	scalp folliculitis	ni	Negative	E, L
73	67	M	scalp folliculitis	ni	Negative	Fucidin
74	53	M	scalp folliculitis	12	Positive	F, E, Cl+timodine
75	33	M	scalp folliculitis	ni	ni	Dermovate, hydrochloroquine
76	40	M	scalp folliculitis	240	Positive	F, E, Cl
77	19	M	Acne nuchae keloidalis	ni	ni	E, I
78	41	M	Dissecting scalp folliculitis	ni	Positive	E
79	42	F	scalp folliculitis	ni	Positive	D
80	52	M	scalp folliculitis	240	Negative	L

Supplementary table 2. Summary characteristics of patients.

FD: Folliculitis decalvans; C: Clindamycin; D: Doxycycline; E: Erythromycin; F: Flucloxacillin; I: Isotretinoin; L: Lymecycline; M: Minocycline; O: Oxytetracycline; R: Rifampicin; S: Septrin (Co-trimoxazole); T: Trimethoprim; ni= not identified from medical notes.