BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-022938
Article Type:	Research
Date Submitted by the Author:	03-Apr-2018
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Keywords:	acne vulgaris, antibiotics, antibiotic susceptibility, Propionibacterium acnes

Antibiotic susceptibility of *Propionibacterium acnes* isolated from patients with acne in a public hospital in southwest China

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Running title: Antibiotic susceptibility of *P. acnes* in China

Abstract

Objective: Antibiotics have been routinely used for several decades against *Propionibacterium acnes* (*P. acnes*), but antibiotic resistance of *P. acnes* is becoming a global problem. Only one Chinese study is available. The aim of the study was to investigate the antibiotics used for treating acne in southwest China and evaluate the prevalence and antibiotic susceptibility rates of *P. acnes*.

Design: This was a prospective cross-sectional study. Cutaneous samples were obtained from acne lesions on the face of 375 patients. Samples were cultured in anaerobic medium to identify the presence of *P. acnes*. Susceptibility tests of isolated *P. acnes* were performed for tetracycline, doxycycline, clindamycin, erythromycin, azithromycin, and clarithromycin using the E-test.

Results: *P. acnes* was isolated from 227 patients; 224 isolates (98.7%) were susceptible to doxycycline and 220 (96.9%) were susceptible to tetracycline, followed by clindamycin and clarithromycin in 101 (44.5%) and 102 (44.93%) isolates, respectively. Susceptibility of *P. acnes* was detected for erythromycin in 96 (42.3%) patients, followed by azithromycin in 94 (41.4%). Subjects who received antibiotics (topical and oral) had higher frequencies of antibiotic-resistant *P. acnes* as well as increases antibiotics MICs compared with patients without antibiotics.

Conclusions: *P. acnes* was highly sensitive to cyclines (doxycycline and tetracycline). *P. acnes* showed higher resistance rates to MLS (macrolides-lincosamides-streptogramins) antibiotics (such as erythromycin, azithromycin, clarithromycin, and clindamycin). The irrational use of antibiotics for acne treatment is probably a problem in China and elsewhere. These results suggest that dermatologists should be more prudent in prescribing antibiotics for acne.

Keywords: acne vulgaris; antibiotics; antibiotic susceptibility; *Propionibacterium acnes*; China.

Strengths and limitations of this study

- The study sample was representative of the population of patients with acne.

- The sample size was small and the subjects were from a single center.

- The cross-sectional designed prevented the determination of any cause-to-effect relationship.

Introduction

Acne is one of the most common skin disorders throughout the world, affecting 67-95% of adolescents [1]. Actually, acne is clearly a chronic inflammatory skin disease and not primarily an infectious disease. It is characterized by pleomorphic lesions including comedones, pustules, papules, nodules, and cysts [2]. Its pathogenesis is multifactorial and includes abnomoral sebum secretion, follicular hyperkeratinization, *Propionibacterium acnes* (*P. acnes*) hypercolonization, inflammation, and immunity [3].

P. acnes plays a vital role in the pathogenesis of acne by activating the innate and adaptive immunity. Chemotactic factors and proinflammatory cytokines are produced by immune reactions, resulting in local inflammation and potential scarring [4]. Anti-inflammatory and antimicrobial medications are the basis of acne therapy. Therefore, antibiotics are widely used in acne patients, inhibiting or eradicating the *P. acnes* colonization, and reducing the production of proinflammatory mediators. Topical and systemic antibiotics are frequently used in the treatment of acne [5]. For the past 30 years, a decrease in the percentage of susceptibility of *P. acnes* strains to these antibiotics has been reported in many countries, indicating that antibiotic-resistant *P. acnes* among acne patients is an international problem [6-13].

With the routine and long-term using antibiotics, the resistance profile of *P. acnes* has gradually altered and varies greatly from one region to another. In China, antimicrobial resistance is generally a severe problem, but the treatment of acne with antibiotics (both topical and systemic) is a common practice. Apart from one multicenter cross-sectional observational study [13], little is known about antibiotic-resistant *P. acnes* among patients with acne in China. Therefore, the aim of the present study was to investigate the antibiotics used

for treating acne in southwest China and evaluate the prevalence and antibiotic susceptibility rates of *P. acnes*. These results could help optimize therapeutic strategies for acne in China.

Methods

Patient and Public Involvement

The patients were not involved when the study was designed. Nevertheless, antimicrobial resistance is a raising public concern in China and the present study helps provide some new data that could be used to better understand the resistance of *P. acnes* and improve the treatment strategies. Patients were informed of the purpose and design of the present study when they first came to the hospital for outpatient visits. Samples were taken after the patients were determined to meet the inclusion and exclusion criteria and consented to participate in the study. The patients were informed of the study results through WeChat (a social network app in China) or phone call.

Patients

This was a prospective study. Patients with acne vulgaris attending the Dermatology outpatient clinic of The First Affiliated Hospital of Kunming Medical University were consecutively enrolled between September 2015 and July 2017. The inclusion criteria were: 1) 12-50 years of age; and 2) mild to severe acne vulgaris [14]. The exclusion criteria were: 1) isotretinoin therapy or receiving topical or systemic antibiotics; or 2) other facial skin disease. The washout period was the period considered necessary for the disappearance of the efficacy of topical or systemic treatment according to the half-life of drug: 1 month for topical antibiotics and 1 month for systemic treatment (at least 2 months for isotretinoin therapy). All patients matching the criteria during the study period were asked to participate

in this study.

Basic clinical information (including age, gender, age of onset, and duration of disease) were obtained at the time of patients' entry into the study or subsequently retrieved from the consultation records.

The study was approved by the ethics committee of the Kunming Medical University of China. Prior to the initiation of the study, informed consents were obtained from the patients. When patients were <18 years, informed consents were also obtained from their parents.

Specimen collection, culture, and *P. acnes* identification

Acne lesions were squeezed using a comedo extractor, put into a 1.5 mL sterile anaerobic tube (MCT-150-C, Axygen, Corning, Tewkesbury, MA, USA) and sent to the central laboratory of Dermatology within half an hour. Six samples were taken from each patient. The samples were inoculated into Brucellar blood agar medium supplemented with vitamin K and incubated anaerobically at 35°C for 7 days. *P. acnes* was identified using the VITEK2 system (BioMerieux, Marcy-L'Etoile, France). The pure strains of *P. acnes* were stored at -80°C.

Antibiotic susceptibility testing and determination of MIC

The minimum inhibitory concentrations (MIC) were detected by the Epsilometer test (E-test) method using E-trips (AB Biodisk, Solna, Sweden). The E-strip is a plastic strip with the MIC interpretative on one side and a predefined antibiotic in gradient concentration (totally 29 concentrations, ranging 0.016-256 μg/mL) on the other side. A susceptibility test was performed on Brucella agar using six E-strips (tetracycline, doxycycline, clindamycin, erythromycin, azithromycin, and clarithromycin). All antibiotics were from BioMerieux (Marcy-L'Etoile, France). The E-test MIC is defined as the point on the scale at which the

ellipse of growth inhibition intercepts the strip. Interpretation of the results was done according to the recommendations given by the Clinical and Laboratory Standards Institute (CLSI) as susceptibility and resistance [15]. The MICs of the six antibiotics were tetracycline ≤4 μg/mL, doxycycline ≤4 μg/mL, clindamycin ≤2 μg/mL, erythromycin ≤0.5 μg/mL, azithromycin ≤0.5 μg/mL, and clarithromycin ≤0.5 μg/mL.

Relationship between MIC and patients' treatment history

We compared the MIC to different antibiotics in the condition of topical use and oral administration. To further analyze the correlation between MIC to various antibiotics and treatment history, the patients were divided into three groups, namely antibiotic use group, non-antibiotic use (with other medications or new treatments) group, and without previous therapy group. The other medications frequently used for treating acne were retinoids, traditional Chinese medicine (TCM), and benzoyl peroxide (BPO). The new treatments included intense pulsed light (IPL), blue and red light, photodynamic therapy (PDT), radiofrequency (RF), and alpha hydroxy acid (AHA) [16-20].

Statistical Analysis

SPSS 22.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Continuous variables were expressed as median (interquartile range) and compared using the Mann-Whitney U test for two groups or the Kruskal-Wallis and the post hoc rank-sum test for more than two groups. Categorical variables were expressed as frequencies and percentages and compared using the Chi-square test. Two-sided P-values <0.05 were considered statistically significant.

Results

Baseline characteristics and history of treatment

Samples were taken from acne lesions of 375 acne patients (224 females and 151 males). The patients were 12 to 46 years of age (mean, 22.3 years). Two hundred and twenty-seven strains of *P. acnes* were isolated from the samples, while 148 samples did not yield any growth and were excluded. Among these 227 patients, there were 26 with topical antibiotic topical use only, 67 with oral antibiotics, 53 with no antibiotics but with other treatment, and 81 without any previous therapy (Table 1). There are 93 subjects having received previous antibiotic therapy, accounting for 63.7% (93/146) of patients who have received previous therapy. The topical antibiotics they received included clindamycin (n=19), erythromycin (n=5), and fusidic (n=5). The oral antibiotics they received included azithromycin (n=14), clarithromycin (n=23), minocycline (n=9), doxycycline (n=16), tetracycline (n=2), and roxithromycin (n=10). Other medications included retinoids (n=53), TCM (n=52), and BPO (n=39). New treatments were given in 41 patients and included IPL, blue light, red light, RF, and AHA. The exact treatment history is shown in Table 2.

Results of antibiotic susceptibility testing

When comparing the various antibiotic susceptibilities of *P. acnes* isolated from patients with different antibiotics history (Table 3), most *P. acnes* isolates were susceptible to doxycycline and tetracycline, whereas *P. acnes* had high resistance to other antibiotics. Patients with non-antibiotic treatment and no previous therapy showed similar results, i.e. that *P. acnes* was highly susceptible to doxycycline and tetracycline.

***P. acnes* minimal inhibitory concentration (MIC) differences of subjects in relation to various previous therapies**

There were no obvious differences for MIC medians between topical and systemic antibiotic groups (Table 4). Compared with the previous antibiotic group (topical and oral), the non-antibiotic treatment and no treatment groups showed low levels of *P. acnes* MIC. Moreover, *P. acnes* MICs of the no previous antibiotic therapy group were near or equivalent to those of the no therapy group (Table 5).

Discussion

Antibiotics have been routinely used for several decades against *P. acnes*, but antibiotic resistance of *P. acnes* is becoming a global problem. Only one study is available on the subject from China. Therefore, the present study aimed to investigate the antibiotics used for treating acne in southwest China and evaluate the prevalence and antibiotic susceptibility rates of *P. acnes*. The results suggest that *P. acnes* was highly sensitive to cyclines (doxycycline and tetracycline). *P. acnes* showed higher resistance rates to MLS (macrolides-lincosamides-streptogramins) antibiotics (such as erythromycin, azithromycin, clarithromycin, and clindamycin). Taken together, these results indicate that the irrational use of antibiotics for acne treatment is probably a problem in China and elsewhere. Dermatologists should be more prudent in prescribing antibiotics for acne.

P. acnes predominantly inhabits the pilosebaceous unit region. *P. acnes* have been shown to induce the production of IL-1α and modulate the proliferation and differentiation of keratinocytes, as well as the comedo formation [21]. *P. acnes* promotes the secretion of proinflammatory mediators by human keratinocytes, sebocytes, and peripheral blood mononuclear cells via immune reactions [22-24]. Moreover, *P. acnes* have been involved in lipogenesis, thus exacerbating acne inflammation [21, 25]. Taken together, this evidence suggests that *P. acnes* may play significant roles in the pathogenesis of acne.

Actually, antibiotics targeting *P. acnes* have been a major approach of acne treatment for over half a century and are thought to work largely by inhibiting *P. acnes* colonization and hence limiting the inflammatory reaction. Currently, it is estimated that about 60% of all antibiotics prescribed by dermatologists are for acne vulgaris [26]. Nearly 8% of all antibiotics prescribed are thought to be for dermatological indications in the UK [27]. In the USA, the dermatologists prescribe almost 5% of all antibiotics, although they only account for ≤1% of the physician population [28]. Crucially, topical antibiotics are often used in the treatment of mild-to-moderate acne and oral antibiotics tend to be used for the treatment of moderate-to-severe acne [5, 29]. Based on our observation, antibiotics are widely used in our region for acne treatments. Topical and oral antibiotics are conventionally used in the treatment of acne as the first choice. Of subjects who had received acne treatment, nearly 64% had previous antibiotic therapy, even sometimes two kinds of antibiotics used simultaneously. It is noteworthy that the data regarding *P. acnes* susceptibility to antibiotics from antibiotic for the topical and oral groups are close to each other, highlighting that antibiotic resistance is as serious with the use of topical antibiotics as with oral antibiotics. Dermatologists should be cautious when prescribing antibiotics, regardless of the method of administration.

Antibiotic resistance is a global issue and "antimicrobial resistance is a ticking time bomb not only for the UK but also for the world" [30]. The overuse and misuse of antibiotics play an important part in the development of antibiotic resistance [31]. Thereby, the adequate and reasonable antibiotic use will decrease antibiotic resistance. In fact, there is a huge antibiotic use in dermatology despite limited information on the use of antibiotics for acne. At present, persons with acne usually take prolonged courses (3-6 months) of a single antibiotic, leading to exposure at different concentrations and potentiate resistance [32, 33]. It is necessary to realize that, indeed, antibiotics alleviate acne symptoms to some extent, but resistance, cross-resistance, and topical antibiotic failure are consequences of antibiotic use in

treating acne [5, 11, 34]. It was reported that the combination with retinoids or BPO therapy will improve antibiotics resistance situation compared with antibiotics as single therapy [35-38]. Therefore, the Global Alliance to Improve Outcome in Acne Group recommended nine easy-to-follow suggestions to limit antimicrobial resistance of *P. acnes*, which mainly include: 1) combination of topical retinoid plus antimicrobial as first-line therapy; 2) antibiotics should not be used as monotherapy; 3) avoid the combination of oral and topical antibiotics; 4) concurrent use of benzoyl peroxide-containing product; 5) limit antibiotics use to short periods; discontinue when there is only slight improvement or no further improvement; 6) oral antibiotics should reasonably be used for 3 months; 7) do not switch antibiotics without adequate justification; 8) avoid antibiotics as maintenance therapy; and 9) use topical retinoids for maintenance therapy, with benzoyl peroxide added when necessary [5].

According to the above-mentioned guidelines, the present observational study indicated that there is irrationality in antibiotic use for many of our acne patients. For instance, antibiotics were used as monotherapy and there was concurrent use of oral and topical antibiotics. Clindamycin was the most common topical antibiotic received by our patients. In fact, clindamycin monotherapy is on the low end of the acne efficacy spectrum, and there is some evidence that clindamycin shares a similar effects as that of the vehicle [39]. Macrolides (roxythromycin, azithromycin, and clarithromycin) are more frequently used than cyclines (minocycline, doxycycline, and tetracycline). Actually, MLS (macrolides-lincosamides-streptogramins) antibiotics show higher resistance rates than cyclines.

With an increased use of various antibiotics, emergence of antibiotic resistance in *P. acnes* has gradually become a worldwide problem. In the 1970s, topical antibiotics resistance of *P. acnes* was first reported in the USA. Since then, numerous studies about the antibiotic resistance of *P. acnes* and MICs of frequently used antibiotics demonstrated that high resistance levels and higher MICs are now observed. In Spain, the prevalence of resistant

strains to one antibiotic has been reported to be 94% [34]. In addition, a study in the UK showed definite increases of antibiotic resistant *P. acnes* strains from 34.5% in 1991 to 64% in 1997 [40]. In recent years, a Japanese study showed that resistance of *P. acnes* to antibiotics increased with acne severity [12]. Another study provided evidence of a correlation between the development of antibiotic-resistant *P. acnes* and a longer duration of antibiotic treatment, a longer duration of acne, and increased age [11]. In Korea, patients with a treatment history with topical or oral antibiotics had higher MIC to doxycycline [41]. Data from the only previous study in China about resistance of *P. acnes* showed that macrolides and lincomycin face a serious resistance state [13]. The present study suggests that the use antibiotics (topical and oral) may increase the possibilities of antibiotic-resistant *P. acnes* as well as increase the MICs of antibiotics. The present cross-sectional study showed that antibiotics use will increase the chances of antibiotics resistance and elevate MIC, especially for MLS antibiotics, aggravating antibiotic-resistant strains, compared with subjects without antibiotics for acne. Importantly, we showed that other medicine or new treatments but without antibiotics will not promote antibiotic resistance and alter MIC levels, showing similar results to that of patients without treatment history for acne. Therefore, alternatives to antibiotics in the treatment of acne do not increase the antibiotic susceptibility of *P. acnes* and their MIC [41].

The present study is not without limitations. The sample size was relatively small and the subjects were from a single center. In addition, the cross-sectional designed prevented the determination of any cause-to-effect relationship. Additional multicenter studies are necessary to examine adequately the issue of antibiotics resistance of *P. acnes*.

Conclusions

In conclusion, antibiotics have been used for acne treatment for several decades and antibiotic resistance of *P. acnes* is a result of antibiotics use in the treatment acne. *P. acnes* was highly sensitive to cyclines (doxycycline and tetracycline). *P. acnes* showed higher resistance rates to MLS antibiotics (such as erythromycin, azithromycin, clarithromycin, and clindamycin). The irrational use of antibiotics for acne treatment is probably a problem in China and elsewhere. These results suggest that dermatologists should be more prudent in prescribing antibiotics for acne. It is time to examine the combination and alternative therapy for antibiotics. New devices (IPL/RF/PDT) are now widely accepted by acne patients for safety, convenience, and effectiveness. Future studies should examine these alternatives.

Acknowledgments

We thank all the laboratory staff of the central laboratory of Dermatology.

Data sharing statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.278cr0g. Encoding: 1=topical use; 2=oral administration; 3=no antibiotic use; 4=without previous therapy.

Funding

This work was supported by the National Science Foundation of China (no. 81460469 and 81760559).

Conflict of interests

All authors declare that they have no any conflict of interests.

Authors' contribution

WYZ and QSW carried out the studies, participated in collecting data, and drafted the manuscript. DJS, JJL and YL performed the statistical analysis and participated in its design. TTZ, LH and WJW helped to draft the manuscript. All authors read and approved the final manuscript.

References

1 Ghodsi SZ, Orawa H, Zouboulis CC. Prevalence, severity, and severity risk factors of acne in high school pupils: a community-based study. *J Invest Dermatol* 2009;**129**:2136-41.

2 Strauss JS, Krowchuk DP, Leyden JJ, et al. Guidelines of care for acne vulgaris management. *J Am Acad Dermatol* 2007;**56**:651-63.

3 Zouboulis CC, Eady A, Philpott M, et al. What is the pathogenesis of acne? *Exp Dermatol* 2005;**14**:143-52.

4 Dessinioti C, Katsambas AD. The role of Propionibacterium acnes in acne pathogenesis: facts and controversies. *Clin Dermatol* 2010;**28**:2-7.

5 Thiboutot D, Gollnick H, Bettoli V, et al. New insights into the management of acne: an update from the Global Alliance to Improve Outcomes in Acne group. *J Am Acad Dermatol* 2009;**60**:S1-50.

6 Gonzalez R, Welsh O, Ocampo J, et al. In vitro antimicrobial susceptibility of Propionibacterium acnes isolated from acne patients in northern Mexico. *Int J Dermatol* 2010;**49**:1003-7.

7 Moon SH, Roh HS, Kim YH, et al. Antibiotic resistance of microbial strains isolated from Korean acne patients. *J Dermatol* 2012;**39**:833-7.

8 Nakase K, Nakaminami H, Noguchi N, et al. First report of high levels of clindamycin-resistant Propionibacterium acnes carrying erm(X) in Japanese patients with acne vulgaris. *J Dermatol* 2012;**39**:794-6.

9 Schafer F, Fich F, Lam M, et al. Antimicrobial susceptibility and genetic characteristics of Propionibacterium acnes isolated from patients with acne. *Int J Dermatol* 2013;**52**:418-25.

10 Mendoza N, Hernandez PO, Tyring SK, et al. Antimicrobial susceptibility of Propionibacterium acnes isolates from acne patients in Colombia. *Int J Dermatol* 2013;**52**:688-92.

11 Luk NM, Hui M, Lee HC, et al. Antibiotic-resistant Propionibacterium acnes among acne patients in a regional skin centre in Hong Kong. *J Eur Acad Dermatol Venereol* 2013;**27**:31-6.

12 Nakase K, Nakaminami H, Takenaka Y, et al. Relationship between the severity of acne vulgaris and antimicrobial resistance of bacteria isolated from acne lesions in a hospital in Japan. *J Med Microbiol* 2014;**63**:721-8.

13 Fan Y, Hao F, Wang W, et al. Multicenter cross-sectional observational study of antibiotic resistance and the genotypes of Propionibacterium acnes isolated from Chinese patients with acne vulgaris. *J Dermatol* 2016;**43**:406-13.

14 Pochi PE, Shalita AR, Strauss JS, et al. Report of the Consensus Conference on Acne Classification. Washington, D.C., March 24 and 25, 1990. *J Am Acad Dermatol* 1991;**24**:495-500.

15 *Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty first informational supplement. CLSI document M100-S21*. Wayne: CLSI, 2011.

16 Patidar MV, Deshmukh AR, Khedkar MY. Efficacy of Intense Pulsed Light Therapy in the Treatment of Facial Acne Vulgaris: Comparison of Two Different Fluences. *Indian J Dermatol* 2016;**61**:545-9.

17 Kwon HH, Lee JB, Yoon JY, et al. The clinical and histological effect of home-use, combination blue-red LED phototherapy for mild-to-moderate acne vulgaris in Korean patients: a double-blind, randomized controlled trial. *Br J Dermatol* 2013;**168**:1088-94.

18 Song BH, Lee DH, Kim BC, et al. Photodynamic therapy using chlorophyll-a in the treatment of acne vulgaris: a randomized, single-blind, split-face study. *J Am Acad Dermatol* 2014;**71**:764-71.

19 Barbaric J, Abbott R, Posadzki P, et al. Light therapies for acne. *Cochrane Database Syst Rev* 2016;**9**:CD007917.

20 Lee KR, Lee EG, Lee HJ, et al. Assessment of treatment efficacy and sebosuppressive effect of fractional radiofrequency microneedle on acne vulgaris. *Lasers Surg Med* 2013;**45**:639-47.

21 Isard O, Knol AC, Aries MF, et al. Propionibacterium acnes activates the IGF-1/IGF-1R system in the epidermis and induces keratinocyte proliferation. *J Invest Dermatol* 2011;**131**:59-66.

22 Thiboutot DM, Layton AM, Anne Eady E. IL-17: a key player in the P. acnes inflammatory cascade? *J Invest Dermatol* 2014;**134**:307-10.

23 Kim J. Review of the innate immune response in acne vulgaris: activation of Toll-like receptor 2 in acne triggers inflammatory cytokine responses. *Dermatology* 2005;**211**:193-8.

24 Kistowska M, Gehrke S, Jankovic D, et al. IL-1beta drives inflammatory responses to propionibacterium acnes in vitro and in vivo. *J Invest Dermatol* 2014;**134**:677-85.

25 Isard O, Knol AC, Castex-Rizzi N, et al. Cutaneous induction of corticotropin releasing hormone by Propionibacterium acnes extracts. *Dermatoendocrinol* 2009;**1**:96-9.

26 Del Rosso JQ, Leyden JJ, Thiboutot D, et al. Antibiotic use in acne vulgaris and rosacea: clinical considerations and resistance issues of significance to dermatologists. *Cutis* 2008;**82**:5-12.

27 Clark C. Antibiotic use for acne reducing effectiveness elsewhere, says leading dermatologist. *Pharm J* 2014;**293**:7820-21.

28 Jesitus J. *Dermatologists contribute to overuse of antibiotics. Dermatology Times, Oct 1, 2013.* *http://dermatologytimes.modernmedicine.com/dermatology-times/news/dermatologists-contribute-overuse-antibiotics?page=full (accessed Jan 11, 2016)*, 2013.

29 Dreno B, Thiboutot D, Gollnick H, et al. Antibiotic stewardship in dermatology: limiting antibiotic use in acne. *Eur J Dermatol* 2014;**24**:330-4.

30 Davies SC. *Annual report of the Chief Medical Officer. Volume two, 2011. Infections and the rise of antimicrobial resistance*. London: Department of Health, 2011.

31 *Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2013*. Atlanta: Centers for Disease Control and Prevention, 2013.

32 Thiboutot D, Dreno B, Gollnick H, et al. A call to limit antibiotic use in acne. *J Drugs Dermatol* 2013;**12**:1331-2.

33 Lee YH, Liu G, Thiboutot DM, et al. A retrospective analysis of the duration of oral antibiotic therapy for the treatment of acne among adolescents: investigating practice gaps and potential cost-savings. *J Am Acad Dermatol* 2014;**71**:70-6.

34 Ross JI, Snelling AM, Carnegie E, et al. Antibiotic-resistant acne: lessons from Europe. *Br J Dermatol* 2003;**148**:467-78.

35 Eady EA, Bojar RA, Jones CE, et al. The effects of acne treatment with a combination of benzoyl peroxide and erythromycin on skin carriage of erythromycin-resistant propionibacteria. *Br J Dermatol* 1996;**134**:107-13.

36 Eady EA, Farmery MR, Ross JI, et al. Effects of benzoyl peroxide and erythromycin alone and in combination against antibiotic-sensitive and -resistant skin bacteria from acne patients. *Br J Dermatol* 1994;**131**:331-6.

37 Nast A, Dreno B, Bettoli V, et al. European evidence-based (S3) guidelines for the treatment of acne. *J Eur Acad Dermatol Venereol* 2012;**26 Suppl 1**:1-29.

38 Dreno B, Bettoli V, Ochsendorf F, et al. An expert view on the treatment of acne with systemic antibiotics and/or oral isotretinoin in the light of the new European recommendations. *Eur J Dermatol* 2006;**16**:565-71.

39 *Sanofi Aventis. BenzaClin full prescribing information. 2013. http://medlibrary.org/lib/rx/meds/benzaclin-3/page/3/ (accessed Jan 10, 2016)*, 2013.

40 Coates P, Vyakrnam S, Eady EA, et al. Prevalence of antibiotic-resistant propionibacteria on the skin of acne patients: 10-year surveillance data and snapshot distribution study. *Br J Dermatol* 2002;**146**:840-8.

41 Song M, Seo SH, Ko HC, et al. Antibiotic susceptibility of Propionibacterium acnes isolated from acne vulgaris in Korea. *J Dermatol* 2011;**38**:667-73.

Table 1. Baseline characteristics of the acne patients

Characteristics	Acne patients (n=227)
Age (years), n (%)	
<25	156 (68.7)
>25	71 (31.3)
Gender, n (%)	
Male	93 (41.0)
Female	134 (59.0)
Age at onset (years), n (%)	
<15	65 (28.6)
15-25	143 (63.0)
>25	19 (8.4)
Duration of disease (years), n (%)	
<2	46 (20.3)
>2	181 (79.7)

Disease severity, n (%)	
Mild	33 (14.5)
Moderate	138 (60.8)
Severe	56 (24.7)
Antibiotic use, n (%)	93 (41.0)
Topical use	26 (11.5)
Oral administration	67 (29.5)
Non-antibiotic use, n (%)	53 (23.3)
Without previous therapy, n (%)	81 (35.7)

Table 2. Treatment history of antibiotics for topical use and oral administration

Profile, n (%)	Topical use (n=26)	Oral administration (n=67)
Subjects who received only a kind of antibiotic	10 (38.5)	19 (28.4)
Two kinds of antibiotic	3 (11.5)	7 (10.4)
One type of antibiotic use plus TCM products	6 (23.1)	15 (22.4)
One type of antibiotic use plus BPO	2 (7.7)	9 (13.4)
One type of antibiotic use plus retinoids	2 (7.7)	8 (11.9)
One type of antibiotic use plus physical treatments	3 (11.5)	3 (4.5)
One type of oral antibiotic plus one type of topical antibiotic	-	6 (9.0)

TCM, traditional Chinese herbal medicine; BPO, benzoyl peroxide.

Table 3. *P. acnes* susceptibility to antibiotics in each group

Antibiotics, n (%)	Antibiotic use (n=93)		Non-antibiotic use (n=53)	Without previous therapy (n=81)
	Topical use (n=26)	Oral administration (n=67)		
Azithromycin	8 (30.8)	18 (26.9)	27 (50.9)	41 (50.6)
Clarithromycin	9 (34.6)	17 (25.4)	30 (56.6)	46 (56.8)
Clindamycin	7 (26.9)	21 (31.3)	29 (54.7)	44 (54.3)
Erythromycin	7 (26.9)	18 (26.9)	27 (50.9)	44 (54.3)
Doxycycline	25 (96.2)	65 (97.0)	53 (100)	81 (100)
Tetracycline	24 (92.3)	65 (97.0)	52 (98.1)	79 (97.5)

Table 4. MIC differences of various antibiotics for topical use and oral administration against Propionibacterium acne isolates

Antibiotics	Topical use (n=26)	Oral administration (n=67)	P
Azithromycin	0.032 (0.018-0.117)	0.032 (0.016-0.205)	0.652
Clarithromycin	0.047 (0.016-0.125)	0.047 (0.02-0.125)	0.912
Clindamycin	0.047 (0.023-0.5)	0.047 (0.023-0.125)	0.705
Erythromycin	0.032 (0.016-0.125)	0.032 (0.021-0.102)	0.950
Doxycycline	0.158 (0.06-0.283)	0.125 (0.047-0.38)	0.786
Tetracycline	0.315 (0.094-0.5)	0.25 (0.094-0.38)	0.266

MIC, minimum inhibitory concentrations.

Table 5. MIC differences of antibiotics use group, non-antibiotic use group, and without previous therapy group

Antibiotics	Antibiotic use (n=93)	Non-antibiotic use (n=53)	Without previous therapy (n=81)	P
Azithromycin	0.032 (0.016-0.19)	0.023 (0.016-0.032)*	0.023 (0.016-0.032)*	0.050
Clarithromycin	0.047 (0.016-0.125)	0.023 (0.016-0.032)**	0.023 (0.016-0.032)**	0.074
Clindamycin	0.047 (0.023-0.125)	0.023 (0.016-0.032)**	0.023 (0.016-0.043)**	0.021
Erythromycin	0.032 (0.02-0.11)	0.016 (0.016-0.047)*	0.020 (0.016-0.032)*	0.053
Doxycycline	0.125 (0.047-0.38)	0.047 (0.032-0.125)*	0.032 (0.032-0.125)*	<0.001
Tetracycline	0.250 (0.094-0.38)	0.064 (0.047-0.25)**	0.064 (0.047-0.25)**	<0.001

MIC, minimum inhibitory concentration. *P<0.05 vs. antibiotic use group; **P<0.001 vs. antibiotic use group.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	5-6
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	NA

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	7-8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7-8
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	8-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Antibiotic susceptibility of Propionibacterium acnes isolated from patients with acne in a public hospital in southwest China: a prospective cross-sectional study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-022938.R1
Article Type:	Research
Date Submitted by the Author:	02-Aug-2018
Complete List of Authors:	Zhu, Tingting ; First Affiliated Hospital of Soochow University, Department of Dermatology; First Affiliated Hospital of Kunming Medical University, Department of Dermatology Zhu, Wenyi ; First Affiliated Hospital of Kunming Medical University, Department of Dermatology Wang, Qisa ; First Affiliated Hospital of Kunming Medical University, Department of Dermatology He, Li ; First Affiliated Hospital of Kunming Medical University, Department of Dermatology Wu, Wenjuan ; First Affiliated Hospital of Kunming Medical University, Department of Dermatology Liu, Jinju ; First Affiliated Hospital of Kunming Medical University, Department of Dermatology Li, Yan ; First Affiliated Hospital of Kunming Medical University, Department of Dermatology Sun, Dongjie; First Affiliated Hospital of Kunming Medical University, Department of Dermatology
Primary Subject Heading:	Dermatology
Secondary Subject Heading:	Dermatology, Evidence based practice, Patient-centred medicine
Keywords:	acne vulgaris, antibiotics, antibiotic susceptibility, Propionibacterium acnes, China, DERMATOLOGY

Antibiotic susceptibility of *Propionibacterium acnes* isolated from patients with acne in a public hospital in southwest China: a prospective cross-sectional study.

Running title: Antibiotic susceptibility of *P. acnes* in China

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Abstract

Objective: Antibiotics have been routinely used for several decades against *Propionibacterium acnes* (*P. acnes*), but antibiotic resistance of *P. acnes* is becoming a global problem. Only one related Chinese study is available. The aim of this study was to assess the antibiotic susceptibility of *P. acnes* obtained from patients with acne in southwest China.

Design: This was a prospective cross-sectional study. Cutaneous samples were obtained from acne lesions on the face of 375 patients. Samples were cultured in anaerobic medium to identify the presence of *P. acnes*. Susceptibility tests of isolated *P. acnes* were performed for tetracycline, doxycycline, clindamycin, erythromycin, azithromycin, and clarithromycin using the E-test.

Results: *P. acnes* was isolated from 227 patients; 224 isolates (98.7%) were susceptible to doxycycline and 220 (96.9%) were susceptible to tetracycline, followed by clindamycin and clarithromycin in 101 (44.5%) and 102 (44.93%) isolates, respectively. Susceptibility of *P. acnes* was detected for erythromycin in 96 (42.3%) patients, followed by azithromycin in 94 (41.4%). Subjects who received antibiotics (topical and oral) had higher frequencies of antibiotic-resistant *P. acnes* as well as increased antibiotic MICs compared with patients without antibiotic treatment.

Conclusions: *P. acnes* was highly sensitive to cyclines (doxycycline and tetracycline). *P. acnes* showed higher resistance rates to MLS (macrolides-lincosamides-streptogramins) antibiotics (such as erythromycin, azithromycin, clarithromycin, and clindamycin). The

irrational use of antibiotics for acne treatment is probably a problem in China and elsewhere. These results suggest that dermatologists should be more prudent in prescribing antibiotics for acne.

Keywords: acne vulgaris; antibiotics; antibiotic susceptibility; *Propionibacterium acnes*; China.

Strengths and limitations of this study

- The study sample was representative of the population of patients with acne.
- The sample size was small, and the subjects were from a single center.
- The cross-sectional design prevented the determination of any cause effect relationship.

Introduction

Acne is one of the most common skin disorders throughout the world, affecting 67-95% of adolescents [1]. Actually, acne is clearly a chronic inflammatory skin disease and not primarily an infectious disease. It is characterized by pleomorphic lesions, including comedones, pustules, papules, nodules, and cysts [2]. Its pathogenesis is multifactorial and includes abnormal sebum secretion, follicular hyper-keratinization, *Propionibacterium acnes* (*P. acnes*) hyper-colonization, inflammation, and immunity [3]. *P. acnes* is also considered an opportunistic pathogen causing multiple inflammatory diseases (e.g. endophthalmitis, endocarditis, osteomyelitis, sarcoidosis, keratitis, and the SAPHO syndrome) as well as inflammatory ailments after surgery or the implantation of foreign devices, including prosthetic aortic valve, hip and shoulder implants[4].

P. acnes plays a vital role in the pathogenesis of acne by activating the innate and adaptive immunity. Chemotactic factors and proinflammatory cytokines are produced by immune reactions, resulting in local inflammation and potential scarring [5]. Anti-inflammatory and antimicrobial medications are the basis of acne therapy. Therefore, antibiotics are widely used in acne patients, inhibiting or eradicating the *P. acnes* colonization, and reducing the production of proinflammatory mediators. Topical and systemic antibiotics are frequently used in the treatment of acne [6]. For the past 30 years, a decrease in the percentage of susceptibility of *P. acnes* strains to these antibiotics has been reported in many countries, indicating that antibiotic-resistant *P. acnes* among acne patients is a global problem [7-14].

With routine and long-term use of antibiotics, the resistance profile of *P. acnes* has been gradually altered, and varies greatly from one region to another. In China, antimicrobial resistance is generally a severe problem, but acne treatment with antibiotics (both topical and oral) is a common practice. Apart from one multicenter cross-sectional observational study [14],

little is known about antibiotic-resistant *P. acnes* among patients with acne in China. Therefore, this study aimed to evaluate the antibiotic susceptibility of *P. acnes* isolated from acne patients in southwest China. The resulting findings could help optimize therapeutic strategies for acne in southwest China.

MethodsPatients

This was a prospective study. Patients with acne vulgaris attending the Dermatology outpatient clinic of The First Affiliated Hospital of Kunming Medical University were consecutively enrolled between September 2015 and July 2017. Inclusion criteria were: 1) 12-50 years of age; and 2) mild to severe acne vulgaris [15]. Exclusion criteria were: 1) oral or topical antibiotic in the past month; 2) oral isotretinoin in the past two months; 3) other facial skin diseases. The washout period was the period considered necessary for the disappearance of the efficacy of topical or systemic treatment according to the half-life of drug: 1 month for topical antibiotics and 1 month for systemic treatment (at least 2 months for isotretinoin therapy). All patients matching the criteria during the study period were asked to participate in this study.

Basic clinical information (including age, gender, age of onset, and duration of disease) were obtained at the time of patient enrolment or subsequently retrieved from consultation records.

The study was approved by the ethics committee of the Kunming Medical University of China. Prior to the initiation of the study, informed consent was obtained from all patients. When patients were <18 years, informed consent was obtained from parents.

Specimen collection, culture, and *P. acnes* identification

Acne lesions were squeezed using a comedo extractor, put into a 1.5 mL sterile anaerobic tube (MCT-150-C, Axygen, Corning, Tewkesbury, MA, USA) and sent to the central laboratory of Dermatology within half an hour. Six samples were taken from each patient. The samples were inoculated into Brucellar blood agar medium supplemented with vitamin K and incubated anaerobically at 35°C for 7 days. *P. acnes* was identified using the VITEK2 system with the 21348 VITEK 2 Corynebacterium identification card (BioMerieux, Marcy-L'Etoile, France). The pure strains of *P. acnes* were stored at -80°C.

Antibiotic susceptibility testing and MIC determination

Minimum inhibitory concentrations (MICs) were detected by the Epsilometer test (E-test) method using E-trips (AB Biodisk, Solna, Sweden). The E-strip is a plastic strip with the MIC interpretative on one side and a predefined antibiotic in gradient concentration (totally 29 concentrations, ranging 0.016-256 μg/mL) on the other side. A susceptibility test was performed on Brucella agar using six E-strips (tetracycline, doxycycline, clindamycin, erythromycin, azithromycin, and clarithromycin). All antibiotics were from BioMerieux (Marcy-L'Etoile, France), and incubations were performed at 37°C under anaerobic conditions[16]. The E-test MIC is defined as the point on the scale at which the ellipse of growth inhibition intercepts the strip. Data interpretation was performed according to the recommendations given by the Clinical and Laboratory Standards Institute (CLSI) and the National Committee for Clinical Laboratory Standards (NCCLS) as susceptibility and resistance [17]. An MIC below the breakpoint value was defined as susceptibility. Breakpoints for the six antibiotics were tetracycline ≤4 μg/mL, doxycycline ≤4 μg/mL, clindamycin ≤2 μg/mL, erythromycin ≤0.5 μg/mL, azithromycin ≤0.5 μg/mL, and clarithromycin ≤0.5 μg/mL[18].

Relationship between MIC and patients' treatment history

We compared the MICs of different antibiotics between topical use and oral administrations. To further analyze the associations of MIC with various antibiotics and treatment history, the patients were divided into three groups, namely antibiotic (Group 1), non-antibiotic (Group 2; treatment with other medications or new treatments) and no previous therapy (Group 3) groups. Other medications frequently used for treating acne included retinoids, traditional Chinese medicine (TCM), and benzoyl peroxide (BPO). The new treatments included intense pulsed light (IPL), blue and red light, photodynamic therapy (PDT), radiofrequency (RF), and alpha hydroxy acid (AHA) [19-23].

Statistical Analysis

SPSS 22.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Continuous variables were expressed as median (interquartile range) and compared using the Mann-Whitney U test for two groups or the Kruskal-Wallis and the post hoc rank-sum test for more than two groups. Categorical variables were expressed as frequencies and percentages and compared using the Chi-square test. Two-sided P-values <0.05 were considered statistically significant.

Patient and Public Involvement

Patients were not involved in the study design and implementation. Patients were informed of the study results via WeChat (a social network app in China) or phone calls.

Results

Baseline characteristics and treatment history

Samples were taken from acne lesions of 375 acne patients (224 females and 151 males). The patients were 12 to 46 years of age (mean, 22.3 years). Two hundred and twenty-seven strains of *P. acnes* were isolated from the collected samples, while 148 samples yielded no growth and were excluded. Among the 227 patients, 93 were administered antibiotics (Group 1), including 67 and 26 and treated orally (Group 1a) and topically (Group 1b), respectively; 53 cases had no antibiotics but underwent other treatments (Group 2), while 81 had no previous therapy (Group 3) (Table 1). Group 1 represented 63.7% (93/146) of all patients who had previous therapy (Groups 1 and 2). The topical antibiotics used included clindamycin (n=19), erythromycin (n=5), and fusidic (n=5). The oral antibiotics employed included azithromycin (n=14), clarithromycin (n=23), minocycline (n=9), doxycycline (n=16), tetracycline (n=2), and roxithromycin (n=10). Other medications included retinoids (n=53), TCM (n=52), and BPO (n=39). New treatments were administered in 41 patients and included IPL, blue light, red light, RF, and AHA. The exact treatment history is shown in Table 2 and supplementary Table 1.

Antibiotic susceptibility

When comparing the various antibiotic susceptibilities of *P. acnes* isolated from patients with different antibiotic histories (Table 3), most *P. acnes* isolates were susceptible to doxycycline and tetracycline. Patients in Groups 2 and 3 showed similar results as cases in Group 1, i.e. *P. acnes* was highly susceptible to doxycycline (P=0.067) and tetracycline (P=0.664). *P. acnes* showed high resistance to other antibiotics, and this was significantly higher in patients in Groups 1a and 1b in comparison with Groups 2 and 3 (azithromycin, P=0.003; clarithromycin, P<0.001; clindamycin, P=0.001; erythromycin, P<0.001).

***P. acnes* minimal inhibitory concentration (MIC) differences of subjects in relation to various previous therapies**

There were no obvious differences in MIC medians between Groups 1a and 1b (oral and topical antibiotic groups) as shown in Table 4. Compared with Group 1, Groups 2 and 3 showed lower levels of *P. acnes* MIC. Moreover, *P. acnes* MICs in Group 2 were similar to those of Group 3 (Table 5).

Discussion

In China, the common topical drugs for acne treatment include Adapalene, benzoyl peroxide, clindamycin gel, and fusidic acid cream. Adapalene and benzoyl peroxide easily cause skin irritation, e.g. redness and burning, when used for the first time. Besides, due to the tense relationship between doctors and patients in China, many doctors prioritize clindamycin gel or fusidic acid cream, which show no obvious irritation in acne treatment. The aim of this study was to assess whether topical antibiotics would lead to antibiotic resistance. The results showed that both topical and oral antibiotics caused drug resistance, suggesting that while prescribing topical antibiotics for acne, dermatologists run the risk of promoting drug resistance.

As shown above, *P. acnes* was highly sensitive to cyclines (doxycycline and tetracycline). Meanwhile, *P. acnes* showed higher resistance rates to MLS (macrolides-lincosamides-streptogramins) antibiotics (such as erythromycin, azithromycin, clarithromycin, and clindamycin).

P. acnes predominantly inhabits the pilosebaceous unit region. *P. acnes* induces the production of IL-1α and modulates the proliferation and differentiation of keratinocytes, as well as comedo formation [24]. *P. acnes* promotes the secretion of proinflammatory mediators by human keratinocytes, sebocytes, and peripheral blood mononuclear cells via immune

reactions [25-27]. Moreover, *P. acnes* have been involved in lipogenesis, thus exacerbating acne inflammation [22, 28]. Such evidence suggests that *P. acnes* may play significant roles in the pathogenesis of acne.

Indeed, antibiotics targeting *P. acnes* have been a major approach of acne treatment for over half a century and are thought to work largely by inhibiting *P. acnes* colonization, hence limiting inflammatory reactions. Currently, it is estimated that about 60% of all antibiotics prescribed by dermatologists target acne vulgaris [29]. Nearly 8% of all antibiotics prescribed are thought to be for dermatological indications in the UK [30]. In the USA, dermatologists prescribe almost 5% of all antibiotics, although they only account for ≤1% of the physician population [31]. Crucially, topical antibiotics are often used in the treatment of mild-to-moderate acne, and oral antibiotics tend to be used for this purpose as well [6, 32]. Based on our experience, antibiotics are widely used in our region for acne treatment. Topical and oral antibiotics are conventionally used in the treatment of acne as the first choice. Of subjects who had received acne treatment, nearly 64% had previous antibiotic therapy, even sometimes with two kinds of antibiotics used simultaneously. It is noteworthy that the data regarding *P. acnes* susceptibility to antibiotics for the topical and oral antibiotic groups were close, highlighting that antibiotic resistance is as serious with topical antibiotics as oral ones. Therefore, dermatologists should be cautious when prescribing antibiotics, regardless of the method of administration.

As shown above, most *P. acnes* isolates were susceptible to doxycycline and tetracycline in all three patient groups. This phenomenon may be related to the widespread use of macrolide antibiotics (especially for respiratory system infections) and inducible resistance, suggesting that the antibacterial spectrum of macrolides antibiotics was gradually narrowed. Meanwhile, tetracycline and doxycycline are mainly used for acne, skin infections

and sexually transmitted diseases, with a narrower range of use, resulting in lower resistance rate.

Antibiotic resistance is a global issue and "antimicrobial resistance is a ticking time bomb not only for the UK but also for the world" [33]. Overuse and misuse of antibiotics play an important role in the development of antibiotic resistance [34]. Therefore, an adequate and reasonable use of antibiotics would decrease antibiotic resistance. In fact, antibiotics are widely used in dermatology despite limited information on their usefulness for acne. Currently, individuals with acne usually take prolonged courses (3-6 months) of a single antibiotic, leading to exposure at different concentrations and potential resistance [35, 36]. It is necessary to realize that, indeed, antibiotics alleviate acne symptoms to some extent, but resistance, cross-resistance and topical antibiotic failure are consequences of antibiotic use in treating acne [6, 12, 37]. It was reported that combination with retinoids or BPO therapy improves antibiotic resistance compared with antibiotics as single therapy [38-41]. Therefore, the Global Alliance to Improve Outcome in Acne Group recommended nine easy-to-follow points for limiting antimicrobial resistance of *P. acnes*: 1) combination of topical retinoid plus antimicrobial as first-line therapy; 2) antibiotics should not be used as monotherapy; 3) avoid the combination of oral and topical antibiotics; 4) concurrent use of benzoyl peroxide-containing products; 5) limit antibiotic use to short periods; discontinue when there is only slight improvement or no further improvement; 6) oral antibiotics should reasonably be used for 3 months; 7) do not switch antibiotics without adequate justification; 8) avoid antibiotics as maintenance therapy; and 9) use topical retinoids for maintenance therapy, with benzoyl peroxide added when necessary [6].

According to the above-mentioned guidelines, the present observational study indicated that there is irrationality in antibiotic use for many acne patients. For instance, antibiotics were used as monotherapy and concurrent use of oral and topical antibiotics was also

reported. Clindamycin was the most common topical antibiotic received by the patients included in this study. Indeed, clindamycin monotherapy is on the low end of the acne efficacy spectrum, and evidence suggests that clindamycin shares similar effects as the vehicle[42]. Macrolides (roxythromycin, azithromycin, and clarithromycin) are more frequently used than cyclines (minocycline, doxycycline, and tetracycline). Actually, MLS (macrolides-lincosamides-streptogramins) antibiotics show higher resistance rates compared with cyclines.

With increased use of various antibiotics, the emergence of antibiotic resistance in *P. acnes* has gradually become a global problem. In the 1970s, *P. acnes* resistance to topical antibiotics was first reported in the USA. Since then, numerous studies about the antibiotic resistance of *P. acnes* and MICs of frequently used antibiotics have confirmed high resistance levels and higher MICs. In Spain, the prevalence of resistant strains to one antibiotic has been reported to be 94% [37]. In addition, a study in the UK showed definite increases of antibiotic resistant *P. acnes* strains from 34.5% in 1991 to 64% in 1997 [43]. Recently, a Japanese study showed that *P. acnes* resistance to antibiotics increases with acne severity [13]. Another study provided evidence of associations of the development of antibiotic-resistant *P. acnes* with long duration of antibiotic treatment, long course of acne, and elevated age [12]. In Korea, patients with a treatment history of topical or oral antibiotics show higher MICs to doxycycline compared with those without antibiotic administration[44]. Data from the only previous study in China about *P. acnes* resistance showed that macrolides and lincomycin face a serious resistance state [14]. The present cross-sectional study suggests that use of antibiotics (topically and orally) may increase the odds of antibiotic-resistance in *P. acnes*, elevating the MICs of antibiotics, especially MLS antibiotics, and promoting antibiotic-resistant strains. Importantly, we showed that other medicines or new treatments without antibiotics did not promote antibiotic resistance or alter MICs, with similar results to no

treatment history for acne. Therefore, alternatives to antibiotics in the treatment of acne may not alter the antibiotic susceptibility of *P. acnes*[44].

The present study was not without limitations. The sample size was relatively small, and all subjects were from a single center. In addition, the cross-sectional design prevented the determination of any cause effect relationship. Additional multicenter studies are necessary to examine adequately the issue of antibiotic resistance in *P. acnes*. Macrolides, lincomycin and tetracycline antibiotics can affect the rRNA subunit in bacteria. However, we could not generate relevant data; amplification and sequencing of relevant gene fragments involved in bacterial resistance should be performed in the future.

Conclusions

Overall, antibiotics have been used for acne treatment for several decades, and antibiotic resistance of *P. acnes* is a result of antibiotic use in the treatment acne. *P. acnes* was highly sensitive to cyclines (doxycycline and tetracycline). *P. acnes* showed higher resistance rates to MLS antibiotics (such as erythromycin, azithromycin, clarithromycin, and clindamycin). The irrational use of antibiotics for acne treatment is probably a problem in China and elsewhere. These results suggest that dermatologists should be more cautious in prescribing antibiotics for acne. It is time to examine combination and alternative therapy to antibiotics. New devices (IPL/RF/PDT) are now widely accepted by acne patients for safety, convenience, and effectiveness. Future studies should examine such alternatives.

Acknowledgments

We thank all the laboratory staff of the central laboratory of Dermatology.

Data sharing statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.278cr0g. Encoding: 1=topical use; 2=oral administration; 3=no antibiotic use; 4=without previous therapy.

Funding

This work was supported by the National Science Foundation of China (no. 81460469 and 81760559).

Conflict of interests

All authors declare that they have no any conflict of interests.

Authors' contribution

WYZ and QSW carried out the studies, participated in collecting data, and drafted the manuscript. DJS, JJL and YL performed the statistical analysis and participated in its design. TTZ, LH and WJW helped to draft the manuscript. All authors read and approved the final manuscript.

References

1 Ghodsi SZ, Orawa H, Zouboulis CC. Prevalence, severity, and severity risk factors of acne in high school pupils: a community-based study. *J Invest Dermatol* 2009;**129**:2136-41.

2 Strauss JS, Krowchuk DP, Leyden JJ, et al. Guidelines of care for acne vulgaris management. *J Am Acad Dermatol* 2007;**56**:651-63.

3 Zouboulis CC, Eady A, Philpott M, et al. What is the pathogenesis of acne? *Exp Dermatol* 2005;**14**:143-52.

4 Fischer N, Mak TN, Shinohara DB, et al. Deciphering the intracellular fate of Propionibacterium acnes in macrophages. *Biomed Res Int* 2013;**2013**:603046.

5 Dessinioti C, Katsambas AD. The role of Propionibacterium acnes in acne pathogenesis: facts and controversies. *Clin Dermatol* 2010;**28**:2-7.

6 Thiboutot D, Gollnick H, Bettoli V, et al. New insights into the management of acne: an update from the Global Alliance to Improve Outcomes in Acne group. *J Am Acad Dermatol* 2009;**60**:S1-50.

7 Gonzalez R, Welsh O, Ocampo J, et al. In vitro antimicrobial susceptibility of Propionibacterium acnes isolated from acne patients in northern Mexico. *Int J Dermatol* 2010;**49**:1003-7.

8 Moon SH, Roh HS, Kim YH, et al. Antibiotic resistance of microbial strains isolated from Korean acne patients. *J Dermatol* 2012;**39**:833-7.

9 Nakase K, Nakaminami H, Noguchi N, et al. First report of high levels of clindamycin-resistant Propionibacterium acnes carrying erm(X) in Japanese patients with acne vulgaris. *J Dermatol* 2012;**39**:794-6.

10 Schafer F, Fich F, Lam M, et al. Antimicrobial susceptibility and genetic characteristics of Propionibacterium acnes isolated from patients with acne. *Int J Dermatol* 2013;**52**:418-25.

11 Mendoza N, Hernandez PO, Tyring SK, et al. Antimicrobial susceptibility of Propionibacterium acnes isolates from acne patients in Colombia. *Int J Dermatol* 2013;**52**:688-92.

12 Luk NM, Hui M, Lee HC, et al. Antibiotic-resistant Propionibacterium acnes among acne patients in a regional skin centre in Hong Kong. *J Eur Acad Dermatol Venereol* 2013;**27**:31-6.

13 Nakase K, Nakaminami H, Takenaka Y, et al. Relationship between the severity of acne vulgaris and antimicrobial resistance of bacteria isolated from acne lesions in a hospital in Japan. *J Med Microbiol* 2014;**63**:721-8.

14 Fan Y, Hao F, Wang W, et al. Multicenter cross-sectional observational study of antibiotic resistance and the genotypes of Propionibacterium acnes isolated from Chinese patients with acne vulgaris. *J Dermatol* 2016;**43**:406-13.

15 Pochi PE, Shalita AR, Strauss JS, et al. Report of the Consensus Conference on Acne Classification. Washington, D.C., March 24 and 25, 1990. *J Am Acad Dermatol* 1991;**24**:495-500.

16 Nazipi S, Stødkilde-Jørgensen K, Scavenius C, et al. The Skin Bacterium Propionibacterium acnes Employs Two Variants of Hyaluronate Lyase with Distinct Properties. *Microorganisms* 2017;**5**. pii: E57.

17 *Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty first informational supplement. CLSI document M100-S21*. Wayne: CLSI, 2011.

18 National Committee for Clinical Laboratory Standards Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standards, 6th ed. NCCLS M11-A6. Wayne, PA: Clinical and Laboratory Standards Institute, 2004

19 Patidar MV, Deshmukh AR, Khedkar MY. Efficacy of Intense Pulsed Light Therapy in the Treatment of Facial Acne Vulgaris: Comparison of Two Different Fluences. *Indian J Dermatol* 2016;**61**:545-9.

20 Kwon HH, Lee JB, Yoon JY, et al. The clinical and histological effect of home-use, combination blue-red LED phototherapy for mild-to-moderate acne vulgaris in Korean patients: a double-blind, randomized controlled trial. *Br J Dermatol* 2013;**168**:1088-94.

21 Song BH, Lee DH, Kim BC, et al. Photodynamic therapy using chlorophyll-a in the treatment of acne vulgaris: a randomized, single-blind, split-face study. *J Am Acad Dermatol* 2014;**71**:764-71.

22 Barbaric J, Abbott R, Posadzki P, et al. Light therapies for acne. *Cochrane Database Syst Rev* 2016;**9**:CD007917.

23 Lee KR, Lee EG, Lee HJ, et al. Assessment of treatment efficacy and sebosuppressive effect of fractional radiofrequency microneedle on acne vulgaris. *Lasers Surg Med* 2013;**45**:639-47.

24 Isard O, Knol AC, Aries MF, et al. Propionibacterium acnes activates the IGF-1/IGF-1R system in the epidermis and induces keratinocyte proliferation. *J Invest Dermatol* 2011;**131**:59-66.

25 Thiboutot DM, Layton AM, Anne Eady E. IL-17: a key player in the P. acnes inflammatory cascade? *J Invest Dermatol* 2014;**134**:307-10.

26 Kim J. Review of the innate immune response in acne vulgaris: activation of Toll-like receptor 2 in acne triggers inflammatory cytokine responses. *Dermatology* 2005;**211**:193-8.

27 Kistowska M, Gehrke S, Jankovic D, et al. IL-1beta drives inflammatory responses to propionibacterium acnes in vitro and in vivo. *J Invest Dermatol* 2014;**134**:677-85.

28 Isard O, Knol AC, Castex-Rizzi N, et al. Cutaneous induction of corticotropin releasing hormone by Propionibacterium acnes extracts. *Dermatoendocrinol* 2009;**1**:96-9.

29 Del Rosso JQ, Leyden JJ, Thiboutot D, et al. Antibiotic use in acne vulgaris and rosacea: clinical considerations and resistance issues of significance to dermatologists. *Cutis* 2008;**82**:5-12.

30 Clark C. Antibiotic use for acne reducing effectiveness elsewhere, says leading dermatologist. *Pharm J* 2014;**293**:7820-21.

31 Jesitus J. *Dermatologists contribute to overuse of antibiotics. Dermatology Times, Oct 1, 2013. http://dermatologytimes.modernmedicine.com/dermatology-times/news/dermatologists-contribute-overuse-antibiotics?page=full (accessed Jan 11, 2016)*, 2013.

32 Dreno B, Thiboutot D, Gollnick H, et al. Antibiotic stewardship in dermatology: limiting antibiotic use in acne. *Eur J Dermatol* 2014;**24**:330-4.

33 Davies SC. *Annual report of the Chief Medical Officer. Volume two, 2011. Infections and the rise of antimicrobial resistance*. London: Department of Health, 2011.

34 *Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2013*. Atlanta: Centers for Disease Control and Prevention, 2013.

35 Thiboutot D, Dreno B, Gollnick H, et al. A call to limit antibiotic use in acne. *J Drugs Dermatol* 2013;**12**:1331-2.

36 Lee YH, Liu G, Thiboutot DM, et al. A retrospective analysis of the duration of oral antibiotic therapy for the treatment of acne among adolescents: investigating practice gaps and potential cost-savings. *J Am Acad Dermatol* 2014;**71**:70-6.

37 Ross JI, Snelling AM, Carnegie E, et al. Antibiotic-resistant acne: lessons from Europe. *Br J Dermatol* 2003;**148**:467-78.

38 Eady EA, Bojar RA, Jones CE, et al. The effects of acne treatment with a combination of benzoyl peroxide and erythromycin on skin carriage of erythromycin-resistant propionibacteria. *Br J Dermatol* 1996;**134**:107-13.

39 Eady EA, Farmery MR, Ross JI, et al. Effects of benzoyl peroxide and erythromycin alone and in combination against antibiotic-sensitive and -resistant skin bacteria from acne patients. *Br J Dermatol* 1994;**131**:331-6.

40 Nast A, Dreno B, Bettoli V, et al. European evidence-based (S3) guidelines for the treatment of acne. *J Eur Acad Dermatol Venereol* 2012;**26 Suppl 1**:1-29.

41 Dreno B, Bettoli V, Ochsendorf F, et al. An expert view on the treatment of acne with systemic antibiotics and/or oral isotretinoin in the light of the new European recommendations. *Eur J Dermatol* 2006;**16**:565-71.

42 *Sanofi Aventis. BenzaClin full prescribing information. 2013. http://medlibrary.org/lib/rx/meds/benzaclin-3/page/3/ (accessed Jan 10, 2016)*, 2013.

43 Coates P, Vyakrnam S, Eady EA, et al. Prevalence of antibiotic-resistant propionibacteria on the skin of acne patients: 10-year surveillance data and snapshot distribution study. *Br J Dermatol* 2002;**146**:840-8.

44 Song M, Seo SH, Ko HC, et al. Antibiotic susceptibility of Propionibacterium acnes isolated from acne vulgaris in Korea. *J Dermatol* 2011;**38**:667-73.

434 **Table 1.** Baseline characteristics of the acne patients

Characteristics	Acne patients (n=227)
Age (years)	
<25	156 (68.7)
>25	71 (31.3)
Gender	
Male	93 (41.0)
Female	134 (59.0)
Age at onset (years)	
<15	65 (28.6)
15-25	143 (63.0)
>25	19 (8.4)
Duration of disease (years)	
<2	46 (20.3)
>2	181 (79.7)

Disease severity	
Mild	33 (14.5)
Moderate	138 (60.8)
Severe	56 (24.7)
Antibiotic use	93 (41.0)
Topical use	26 (11.5)
Oral administration	67 (29.5)
Non-antibiotic use	53 (23.3)
Without previous therapy	81 (35.7)

Data were expressed as n (%).

Table 2. Treatment history of antibiotics for oral administration (Group 1a) and topical use (Group 1b)

Profile	Group 1a (n=67)	Group 1b (n=26)
Subjects who received only a kind of antibiotic	19 (28.4)	10 (38.5)
Two kinds of antibiotic	7 (10.4)	3 (11.5)
One type of antibiotic use plus TCM products	15 (22.4)	6 (23.1)
One type of antibiotic use plus BPO	9 (13.4)	2 (7.7)
One type of antibiotic use plus retinoids	8 (11.9)	2 (7.7)
One type of antibiotic use plus physical treatments	3 (4.5)	3 (11.5)
One type of oral antibiotic plus one type of topical antibiotic	6 (9.0)	-

Data were expressed as n (%). TCM, traditional Chinese herbal medicine; BPO, benzoyl peroxide.

443 **Table 3.** *P. acnes* susceptibility to antibiotics in each group

Antibiotics, n (%)	Group 1 (n=93)			Group 2 (n=53)	Group 3 (n=81)	P
	Group 1a (n=67)	Group 1b (n=26)	Total (n=93)			
Azithromycin	18 (26.9)	8 (30.8)	26 (28.0)#*	27 (50.9)	41 (50.6)	0.003
Clarithromycin	17 (25.4)	9 (34.6)	26 (28.0)#**	30 (56.6)	46 (56.8)	<0.001
Clindamycin	21 (31.3)	7 (26.9)	28 (30.1)#*	29 (54.7)	44 (54.3)	0.001
Erythromycin	18 (26.9)	7 (26.9)	25 (26.9)#**	27 (50.9)	44 (54.3)	<0.001

Doxycycline	65 (97.0)	25 (96.2)	90 (96.8)	53 (100)	81 (100)	0.067
Tetracycline	65 (97.0)	24 (92.3)	89 (95.7)	52 (98.1)	79 (97.5)	0.664

Data were expressed as n (%). #P<0.05 vs. Group 2; *P<0.05 vs. Group 3; **P<0.001 vs. Group 3.

Table 4. MIC differences of various antibiotics for oral administration (Group 1a) and topical use (Group 1b) against Propionibacterium acne isolates

Antibiotics	Group 1a (n=67)	Group 1b (n=26)	P
Azithromycin	0.032 (0.016-0.205)	0.032 (0.018-0.117)	0.652
Clarithromycin	0.047 (0.02-0.125)	0.047 (0.016-0.125)	0.912
Clindamycin	0.047 (0.023-0.125)	0.047 (0.023-0.5)	0.705
Erythromycin	0.032 (0.021-0.102)	0.032 (0.016-0.125)	0.950
Doxycycline	0.125 (0.047-0.38)	0.158 (0.06-0.283)	0.786
Tetracycline	0.25 (0.094-0.38)	0.315 (0.094-0.5)	0.266

The values were MIC (μg/mL) and were expressed as median (interquartile range). MIC, minimum inhibitory concentrations.

Table 5. MIC differences of three groups

Antibiotics	Group 1 (n=93)	Group 2 (n=53)	Group 3 (n=81)	P
Azithromycin	0.032 (0.016-0.19)	0.023 (0.016-0.032)*	0.023 (0.016-0.032)*	0.050
Clarithromycin	0.047 (0.016-0.125)	0.023 (0.016-0.032)**	0.023 (0.016-0.032)**	0.074
Clindamycin	0.047 (0.023-0.125)	0.023 (0.016-0.032)**	0.023 (0.016-0.043)**	0.021
Erythromycin	0.032 (0.02-0.11)	0.016 (0.016-0.047)*	0.020 (0.016-0.032)*	0.053
Doxycycline	0.125 (0.047-0.38)	0.047 (0.032-0.125)*	0.032 (0.032-0.125)*	<0.001
Tetracycline	0.250 (0.094-0.38)	0.064 (0.047-0.25)**	0.064 (0.047-0.25)**	<0.001

The values were MIC (μg/mL) and were expressed as median (interquartile range). MIC, minimum inhibitory concentration. *P<0.05 vs. Group 1; **P<0.001 vs. Group 1.

Supplementary Table 1. Treatment history of patients with antibiotics administered orally (Group 1a) or topically (Group 1b)

Group 1a	N=67	Group 1b	N=26
Subjects who received only one antibiotic			
Clarithromycin	6 (9.0)	Clindamycin	7 (26.9)
Azithromycin	3 (4.5)	Erythromycin	1 (3.8)
Minocycline	2 (3.0)	Fusidic	2 (7.7)
Doxycycline	5 (7.5)		
Roxithromycin	3 (4.5)		
Two antibiotics			
Clarithromycin+roxithromycin	1 (1.5)	Clindamycin+erythromycin	1 (3.8)
Minocycline+roxithromycin	1 (1.5)	Fusidic+erythromycin	1 (3.8)
Doxycycline+clarithromycin	2 (3.0)	Fusidic+clindamycin	1 (3.8)
Tetracycline+clarithromycin	1 (1.5)		

Azithromycin+doxycycline	1 (1.5)		
Clarithromycin+minocycline	1 (1.5)		
One antibiotic plus TCM products			
Clarithromycin+TCM	5 (7.5)	Clindamycin+TCM	5 (19.2)
Azithromycin+TCM	3 (4.5)	Erythromycin+TCM	1 (3.8)
Doxycycline+TCM	2 (3.0)		
Roxithromycin+TCM	1 (1.5)		
Minocycline+TCM	3 (4.5)		
Tetracycline+TCM	1 (1.5)		
One antibiotic plus BPO			
Doxycycline+BPO	1 (1.5)	Fusidic+BPO	1 (3.8)
Azithromycin+BPO	3 (4.5)	Clindamycin+BPO	1 (3.8)
Clarithromycin+BPO	5 (7.5)		

One antibiotic plus retinoids			
Azithromycin+retinoids	2 (3.0)	Clindamycin+retinoids	2 (7.7)
Doxycycline+retinoids	3 (4.5)		
Roxithromycin+retinoids	3 (4.5)		
One antibiotic plus physical treatment			
Azithromycin+physical treatmens	1 (1.5)	Clindamycin+physical treatment	2 (7.7)
Doxycycline+physical treatments	2 (3.0)	Erythromycin+physical treatment	1 (3.8)
One oral antibiotic plus one topical antibiotic			
Clindamycin+azithromycin	1 (1.5)		
Clindamycin+minocycline	2 (3.0)		
Fusidic+roxithromycin	1 (1.5)		
Clindamycin+clarithromycin	2 (3.0)		

Data were expressed as n (%). TCM, traditional Chinese herbal medicine; BPO, benzoyl peroxide.

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	5-6
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	NA

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	7-8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7-8
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	8-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.