

Effect of Long-Snake Moxibustion on Gut Microbiota of Patients with Ankylosing Spondylitis

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Keywords

Ankylosing spondylitis · Long-snake moxibustion · Gut microbiota · 16S rDNA

Abstract

Objective: The objective was to study the effect of long-snake moxibustion intervention on gut microbiota of patients with ankylosing spondylitis (AS) by 16S rDNA sequencing technology. **Methods:** Thirty AS patients and 30 healthy volunteers were recruited and treated with long-snake moxibustion once a week for 12 weeks. AS patients were divided into pretreatment and posttreatment groups. VAS, BASDAI, and BASFI scores of AS patients before and after treatment were collected. 16S rDNA high-throughput sequencing technology was used to analyze the characteristics and differences of gut microbiota in AS patients before and after treatment and in healthy volunteers. **Results:** VAS, BASDAI, and BASFI scores of AS patients after long-snake moxibustion treatment were lower than those of pretreatment group ($p < 0.05$). The results of gut microbiota alpha diversity showed that Ace and Chao1 index of the posttreatment group were higher than those of the health group ($p < 0.05$), but there was no statistical significance in Ace and Chao1 index between the pretreatment group and the posttreatment group ($p > 0.05$). Beta diversity analysis showed that mild classification aggregation occurred

between the health group and the pretreatment group but did not reach a significant level, and there was no significant difference between the pretreatment group and the posttreatment group. The results of species abundance showed that, at the phylum level, compared with the health group, the relative abundance of Firmicutes and Proteobacteria decreased in the pretreatment group, while the relative abundance of Bacteroidetes and Actinobacteria increased. Compared with the pretreatment group, the relative abundance of Firmicutes increased and the relative abundance of Actinobacteria decreased in the posttreatment group, but there were no statistically significant differences in the above changes ($p > 0.05$). At the genus level, compared with the health group, the relative abundances of Subdoligranulum in the pretreatment group were increased ($p < 0.05$), while the relative abundances of Bifidobacterium and Streptococcus were decreased ($p < 0.05$). Compared with the pretreatment group, the relative abundance of Romboutsia in the posttreatment group was increased ($p < 0.05$). **Conclusion:** Long-snake moxibustion can obviously improve the clinical symptoms of AS patients. The possible mechanism of action is related to regulating the abundance of gut microbiota, increasing beneficial bacteria, and restoring the homeostasis of gut microorganisms.

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Effekt der *long-snake*-Moxibustion auf die Darm-Mikrobiota von Patienten mit ankylosierender Spondylitis

Schlüsselwörter

Ankylosierende Spondylitis · Long-snake Moxibustion · Darm-Mikrobiota · 16S-rDNA

Zusammenfassung

Ziel: Untersuchung des Effekts der Anwendung von *long-snake*-Moxibustion auf die Darm-Mikrobiota von Patienten mit ankylosierender Spondylitis (AS) laut 16S-rDNA-Sequenzierung. **Methoden:** 30 AS-Patienten und 30 gesunde Probanden wurden rekrutiert und 12 Wochen lang einmal wöchentlich mit *long-snake*-Moxibustion behandelt. Die AS-Patienten wurden in eine Gruppe vor und eine Gruppe nach der Behandlung eingeteilt. Die VAS-, BASDAI- und BASFI-Scores der AS-Patienten vor und nach der Behandlung wurden erhoben. Mittels 16S-rDNA-Hochleistungssequenzierung wurden die Eigenschaften und Unterschiede der Darm-Mikrobiota der AS-Patienten vor und nach der Behandlung sowie der gesunden Probanden analysiert. **Ergebnisse:** Die VAS-, BASDAI- und BASFI-Scores der AS-Patienten nach der *long-snake*-Moxibustion waren niedriger als in der Gruppe vor der Behandlung ($p < 0.05$). Die Ergebnisse zur alpha-Diversität der Darm-Mikrobiota zeigten einen höheren ace- und chao1-Index in der Gruppe nach der Behandlung als in der Gruppe der gesunden Probanden ($p < 0.05$), jedoch keinen statistischen Unterschied im ace- und chao1-Index zwischen der Gruppe vor der Behandlung und der Gruppe nach der Behandlung ($p > 0.05$). Die beta-Diversitätsanalyse ergab eine leichte Klassifikations-Aggregation zwischen der Gruppe der gesunden Probanden und der Gruppe vor der Behandlung, die jedoch kein signifikantes Ausmaß erreichte, und auch zwischen der Gruppe vor der Behandlung und der Gruppe nach der Behandlung bestand kein signifikanter Unterschied. Die Ergebnisse zur Abundanz der einzelnen Arten zeigten auf Phylum-Ebene eine verringerte relative Abundanz von Firmicutes und Proteobacteria in der Gruppe vor der Behandlung im Vergleich zur Gruppe der gesunden Probanden, während die relative Abundanz von Bacteroidetes und Actinobacteria erhöht war. In der Gruppe nach der Behandlung war im Vergleich zur Gruppe vor der Behandlung die relative Abundanz von Firmicutes erhöht und die von Proteobacteria verringert, jedoch bestand bei beiden Veränderungen kein statistisch signifikanter Unterschied ($p > 0.05$). Auf Genus-Ebene war die relative Abundanz von Subdoligranulum in der Gruppe vor der Behandlung im Vergleich zur Gruppe der gesunden Probanden erhöht ($p < 0.05$), während die

relative Abundanz von Bifidobacterium und Streptococcus verringert war ($p < 0.05$). In der Gruppe nach der Behandlung war im Vergleich zur Gruppe vor der Behandlung die relative Abundanz von Romboutsia erhöht ($p < 0.05$). **Schlussfolgerung:** Die *long-snake*-Moxibustion kann offensichtlich die klinischen Symptome von AS-Patienten verbessern. Der Wirkungsmechanismus hängt möglicherweise mit der Regulation der Abundanzen der Darm-Mikrobiota im Sinne einer Zunahme der nützlichen Bakterien und Wiederherstellung der Homöostase der Darm-Mikroorganismen zusammen.

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Introduction

Ankylosing spondylitis (AS) is a chronic and progressive arthritis disease, which mainly affects the axial bones and sacroiliac joints. Most patients show severe backache and arthralgia [1, 2]. The progress of the advanced diseases can lead to joint deformity and limited activities, which will reduce the quality of life of patients [3, 4]. The incidence group of AS is mostly young adults aged 20–30. A recent survey found that 30 out of every 10,000 people suffer from AS, and the incidence rate is on the rise [5], which has caused a heavy burden to patients and society. At present, the clinical treatment of AS mainly depends on drug therapy, including nonsteroidal anti-inflammatory drugs, anti-rheumatic drugs, tumor necrosis factor (TNF- α) inhibitors, and biological agents. Although these drugs can improve the symptoms of patients to a certain extent, however, the course of disease cannot be controlled, and there are problems such as large side effects and high cost [6], while traditional Chinese medicine is economical and effective in treating AS, which has great advantages. Long-snake moxibustion is a traditional external treatment method of traditional Chinese medicine. Because of its definite curative effect, safety, comfort, and no side effects, it has attracted more and more attention in clinic. Our previous research found that long-snake moxibustion can significantly activate peroxisome proliferators-activated receptors, inhibit the expression of inflammatory factors such as TNF- α , IL-1 β , IL-6, IL-17, and IL-23, play an anti-inflammatory role, and finally relieve AS [7]. Combined with many current studies, it has been shown that long-snake moxibustion can obviously improve the clinical symptoms of AS patients and regulate the serum inflammatory response and metabolism in vivo [8, 9]. Gut microbiota is considered as the second genome of human beings. It is found that intestinal ecological imbalance in AS patients and the species and abundance of flora have changed [10]. However, there are few studies on the regulation of

Table 1. Comparison of two groups of general data

Groups	Cases	Gender/case		Age/years, mean \pm SD	Course of disease/years, mean \pm SD
		man	woman		
Ankylosing spondylitis subjects	30	15	15	35.33 \pm 8.75	8.43 \pm 5.15
Health subjects	30	15	15	35.06 \pm 8.22	

intestinal microflora of AS patients by long-snake moxibustion. Therefore, this study explored the influence of long-snake moxibustion on gut microbiota of AS patients based on 16s rDNA sequencing technology and provided some experimental basis for the treatment of AS with long-snake moxibustion.

Materials and Methods

Research Object

From September 2020 to June 2022, 30 AS patients (15 males and 15 females, aged 18–50 years) who were clinically diagnosed by acupuncture department of Hongdu Chinese Medicine Hospital in Nanchang City were selected. Observation indexes of long-snake moxibustion before treatment are the pretreatment group, and those after treatment are the posttreatment group. At the same time, 30 healthy volunteers (15 males and 15 females, aged 18–50 years) matched with patients' age were selected as the health group. There was no significant difference in baseline data such as sex and age between the two groups (Table 1) ($p > 0.05$). This research plan was approved by the Nanchang Hongdu Hospital of Traditional Chinese Medicine Medical Ethics Committee (KY-2020-007), and all subjects volunteered to participate and signed the informed consent form.

The diagnosis of the included patients conforms to the diagnostic criteria in the Guidelines for Diagnosis and Treatment of Ankylosing Spondylitis issued by Rheumatology Branch of Chinese Medical Association in 2010: (1) low back pain lasts for at least 3 months, and the pain improves with activities, but the rest does not relieve it; (2) the lumbar spine is limited in the anteroposterior and lateral flexion directions; (3) the range of thoracic expansion is less than the normal value of the same age and gender; (4) bilateral sacroiliitis is grade II ~ IV, or unilateral sacroiliitis is grade III ~ IV. If the patient has (4) with any one of (1–3) attached, it can be diagnosed as AS [11]. Exclusion criteria: (1) pregnant and lactating patients; (2) patients with joint deformity, spinal rigidity, and severe dysfunction in the late stage of the disease; (3) patients with acute complications need to be treated with gluco-

corticoid; (4) patients with other serious primary diseases or mental diseases; (5) patients with peptic ulcer; (6) patients who have taken nonsteroidal anti-inflammatory drugs, anti-rheumatic drugs, TNF- α inhibitors, or biological agents within 3 months.

Therapeutic Method

- Moxibustion treatment area: from Dazhui point (in the spinal region, in the subspinous process depression of the seventh cervical vertebra, on the posterior median line) of governor vessel to Yaoshu point (in the sacral region, just opposite the sacral canal hiatus, on the posterior median line).
- Operation method: the patient lies prone on the treatment bed, with a comfortable posture and bare back. The performer routinely disinfects the spinal region of the patient for three times, smears ginger juice along the spinal column and spreads 2–3 layers of gauze, spreads ginger paste with a width of 4–5 cm and a height of 1–2 cm on the gauze, heap moxa (about 200 g) on ginger paste to form a long serpentine moxa cone with a bottom width of 2–3 cm, a height of 2 cm, a lower width and a top tip. Ignite the head, body, and tail of the moxa cone to make it burn naturally, which is one moxa cone. After burning completely, replace moxa and continue moxibustion for three times. After the treatment, remove the moxa ash and ginger paste, wipe the patient's back with a warm towel, drink a proper amount of warm water after dressing, and rest for 10 min.
- Treatment cycle: once a week, with 4 weeks as a course of treatment, and continuous treatment for 3 courses.
- Matters needing attention: keep the ambient temperature moderate; before moxibustion, patients should avoid overeating, starvation, or strenuous exercise; pay attention to ask the patient's temperature when applying moxibustion, and adjust the moxibustion in time to avoid burns; if burns and scalds occur, they should be disinfected and smeared with scald ointment, and the severe cases should be treated surgically; after moxibustion, ask the patient to take shelter from the wind and cold, and take a bath after 5–6 h.

Table 2. VAS, BASDAI, and BASFI scores of AS patients before and after treatment

Groups	VAS score	BASDAI score	BASFI score
Pretreatment group	6.86±1.40	5.01±1.12	5.04±1.77
Posttreatment group	3.60±1.62*	3.15±1.24*	3.07±1.60*

*Compared with Pretreatment group, $p < 0.05$.

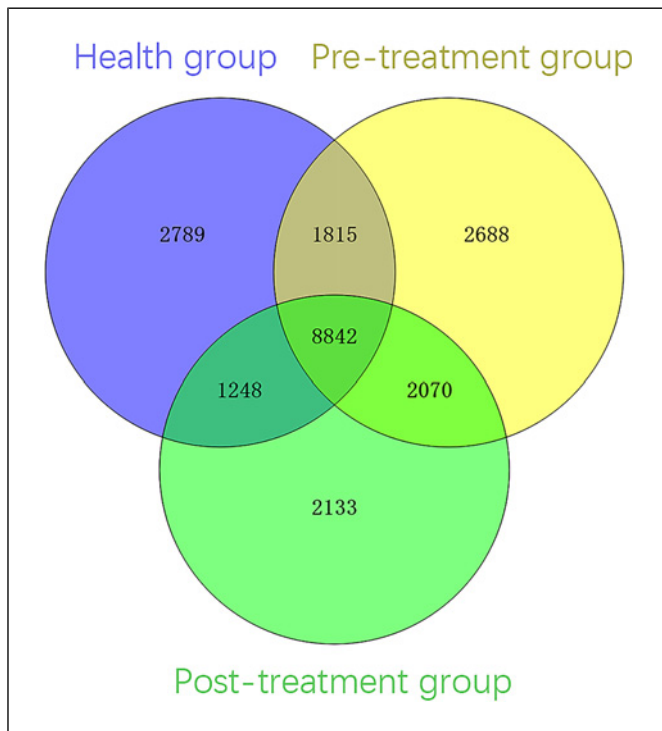


Fig. 1. Venn diagram of OTU number of intestinal flora in each group.

Clinical Symptom Assessment

Visual analogue scale (VAS) [12], Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), and Bath Ankylosing Spondylitis Function Index (BASFI) were used to score AS patients (0–10 points) [13, 14]. The higher the score, the higher the disease activity.

Detection and Analysis of Flora

In the morning, 1 g morning stool samples of healthy volunteers and AS patients were collected before and after treatment and transported to the refrigerator at -80°C on ice within 30 min for storage. 16S rDNA sequencing analysis was carried out on the collected specimens, including fecal DNA extraction and detection, PCR amplification, product purification, library construction, and quality inspection. The library was sequenced by Illumina Miseq/HiSeq2500 platform, and the sequence was clustered into OTUs according to the consistency of ninety-seven percent, and the species annotation was carried out with

Silva database. According to the species annotation, the flora structure and diversity were calculated, the differences between groups were compared, and the different characteristics of sample community structure were analyzed.

Statistical Analysis

Statistical analysis was carried out by SPSS 26.0 statistical software, and the measurement data were expressed by mean standard deviation. If the data followed a normal distribution, a t test was used; if it did not follow a normal distribution, a rank-sum test was employed. A difference was considered statistically significant if $p < 0.05$.

Results

Comparison of Symptom Scores before and after Treatment

After long-snake moxibustion treatment, the scores of VAS, BASDAI, and BASFI of AS patients were significantly lower than those before treatment ($p < 0.05$), as shown in Table 2.

OTU Number of Intestinal Flora

As shown in Figures 1 and 2, 15,415 OTUs were obtained from the three groups, including 14,694 OTUs in the health group, 15,415 OTUs in the pretreatment group and 14,293 OTUs in the posttreatment group, 10,657 OTUs in the health group and the pretreatment group, 10,912 OTUs in the pretreatment group and the post-treatment group, and 10,090 OTUs in the health group and the posttreatment group.

Alpha Diversity Analysis of Intestinal Flora

The alpha diversity index of the three groups of samples is shown in Table 3. Before treatment, the Shannon and Simpson indexes of the group were not statistically different from those of the health group ($p > 0.05$), while the Ace and Chao1 indexes were higher than those of the health group ($p < 0.05$). There was no significant difference in Shannon, Simpson, Ace, and Chao1 between the pretreatment group and the posttreatment group ($p > 0.05$). It is suggested that the richness of intestinal flora in AS patients is higher than that in healthy people, but the treatment of long-snake moxibustion has no obvious effect on it.

Table 3. Comparison of alpha diversity indexes in different groups of samples (mean ± SD)

Groups	Health group	Pretreatment group	Posttreatment group
Shannon index	6.51±0.95	6.88±0.74	6.47±1.30
Simpson index	0.94±0.06	0.95±0.03	0.92±0.08
Ace index	2,894.42±882.56	3,448.87±878.44*	3,432.67±943.70
Chao1 index	2,761.20±824.91	3,284.41±816.05*	3,259.11±890.16

*Compared with Health group, $p < 0.05$.

Beta Diversity Analysis of Intestinal Flora

Based on weighted_unifrac algorithm, the results of PCoA analysis are plotted (Fig. 2a and b). The closer the samples are on the graph, the greater the similarity. As shown in the figure, there is a slight clustering phenomenon between the health group and the pretreatment group, but it has not reached a significant level, and there is no significant difference between the pretreatment group and the posttreatment group.

Analysis of Relative Abundance of Intestinal Flora

According to the histogram of relative abundance of intestinal flora (Fig. 3a, b), at the phylum level, the top five relative abundance distributions of the three groups of intestinal flora are Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Tenericutes; compared with the health group, before treatment, the relative abundance of Firmicutes and Proteobacteria decreased, while the relative abundance of Bacteroides and Actinomycetes increased. Compared with the pretreatment group, the relative abundance of Firmicutes increased and the relative abundance of Actinomycetes decreased in the posttreatment group, but there was no significant statistical difference between the above changes combined with the data in Table 4 ($p > 0.05$). At the genus level, the top five relative abundance distributions of the three groups of intestinal flora are Subdoligranulum, Bacteroides, Faecalibacterium, Romboutsia, and Blaut. According to the data in Table 5, compared with the health group, the relative abundance of Micrococcus rariorum increased ($p < 0.05$), and the relative abundance of Bifidobacterium and Streptococcus decreased ($p < 0.05$) in the pretreatment group. Compared to the pretreatment group, the relative abundance of Roma Boots increased significantly after treatment ($p < 0.05$), while the relative abundance of Eubacterium huaxiensis decreased.

Discussion

The AS belongs to the categories of “arthralgia syndrome” and “rheumatism” in Chinese medicine, and its basic pathogenesis is deficiency of kidney essence and cold-dampness stagnation, which leads to obstruction of meridians, obstruction of qi and blood, malnutrition of

bones and muscles, and arthralgia for a long time. This disease is closely related to governor vessel, for example, “Plain question bone theoretical” records that “The pathological changes of governor vessel will cause the symptoms of stiff spine, and low back pain can’t be shaken,” and another example is “The Union of Heaven and Human Beings,” which says that “Yang qi can’t be opened or closed, and cold comes from it, which leads to great rolls,” and the governor vessel is just the sea of Yang meridian, so the treatment often takes both the symptoms and root causes into consideration, mainly focusing on tonifying kidney and strengthening bones, regulating the governor, and regulating qi, warming yang, and dispelling cold. Long-snake moxibustion is a traditional Chinese medicine external treatment method. Because it is moxibustion with ginger as a spacer on governor vessel, it is also called “Du moxibustion” or “Spreading Moxibustion.” This therapy combines the warming effect of ginger with the warming effect of moxibustion and penetrates into the governor vessel, which can play a therapeutic role in strengthening the true elements, warming the governor vessel, and dredging qi and blood for AS patients.

Gut microbiota is the most diverse and dense microflora in human body [15, 16]. Its main function is to protect the host from pathogens and exert benefits through various mechanisms, which is closely related to the occurrence of various host diseases. Studies have shown that intestinal microflora plays a key role in the development and function of intestinal innate and adaptive immune systems, and dysbacteriosis may lead to the occurrence and progress of autoimmune diseases [17]. The pathogenesis of AS is closely related to inflammatory bowel disease. More than 70% of AS patients have subclinical intestinal inflammation. The inefficient separation of the intestinal cavity from subepithelial space is related to the abnormal activation of immune cells residing in the mucosa. These immune cells are recycled, which may transfer inflammation to joints and establish pathogenic intestinal-articular axis [18]. AS early as 2002, there was the first assessment of the intestinal microflora in AS patients [19], and since then, many studies have been carried out, indicating that the intestinal microflora in AS patients is obviously different from that in healthy people. For example, Costello and others found that compared with healthy control groups, the species

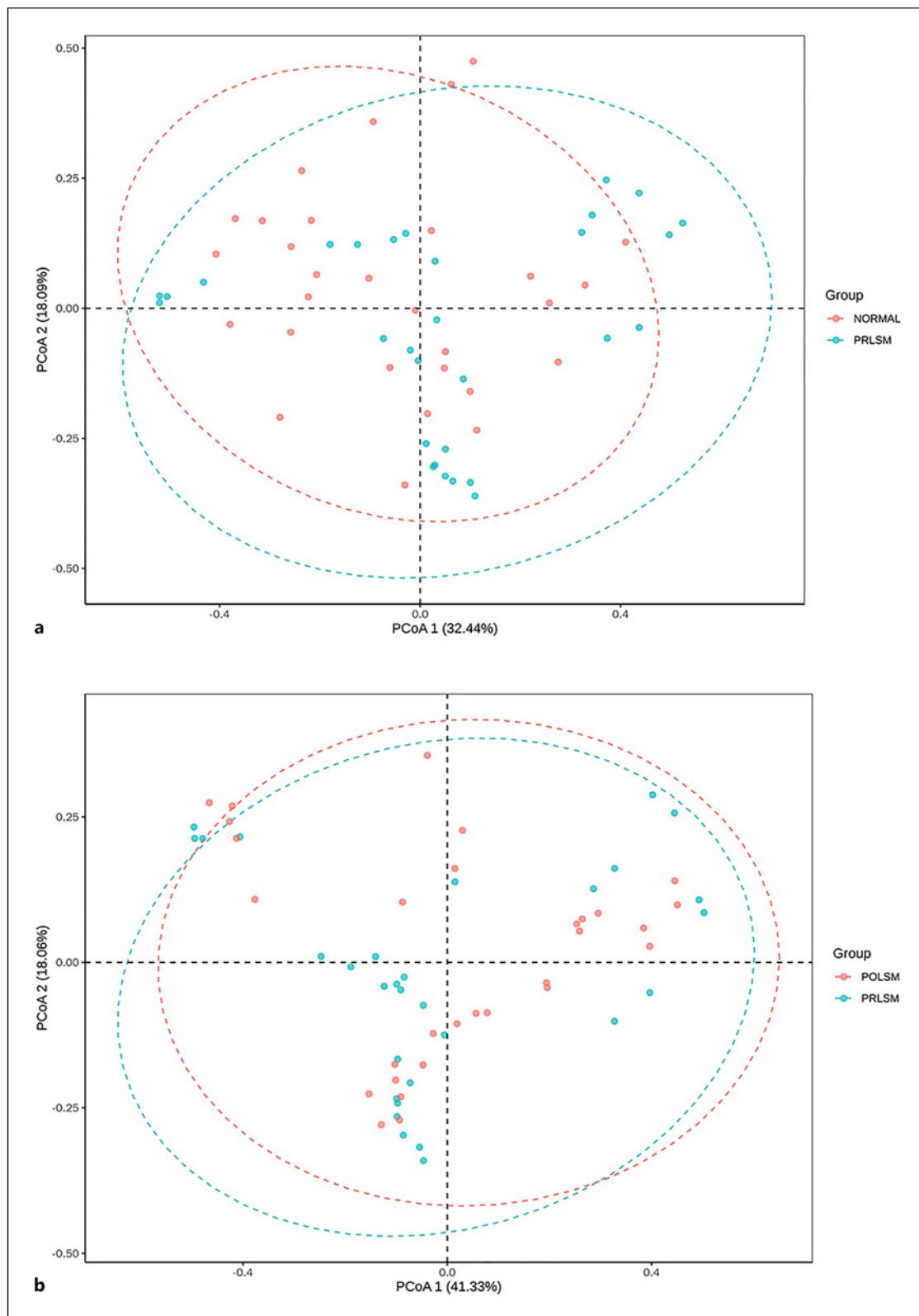


Fig. 2. PCoA analysis result chart. **a** PCoA analysis chart of health group and pretreatment group (NORMAL: health group; PRLSM: pretreatment group). **b** PCoA analysis chart of pretreatment group and posttreatment group (POLSM: posttreatment group).

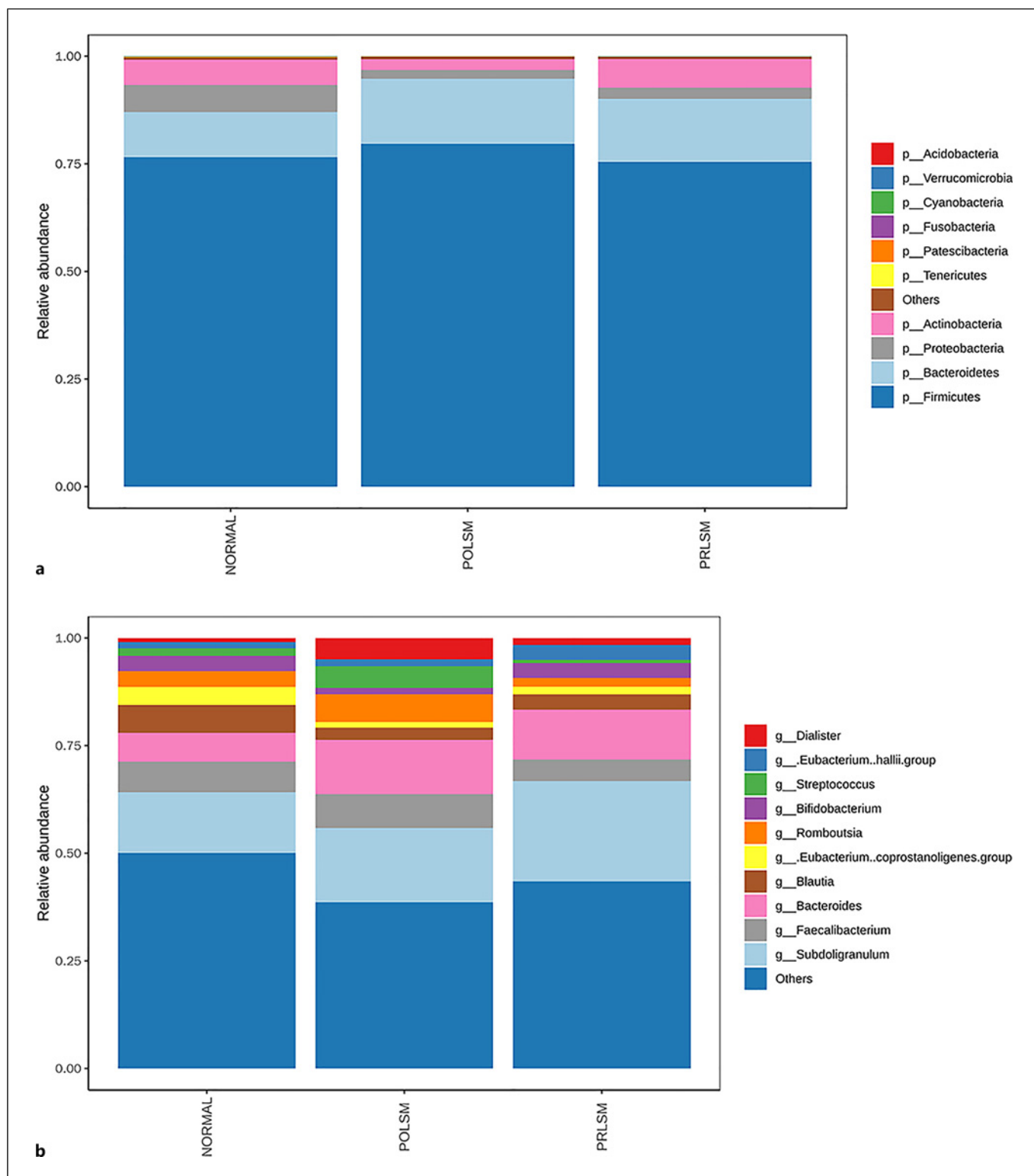


Fig. 3. Histogram of horizontal relative abundance of intestinal flora in each group ((NORMAL: health group; POLSM: posttreatment group; PRLSM: pretreatment group). **a** Histogram of relative abundance at phylum level. **b** Horizontal relative abundance histogram of genus.

abundance of intestinal microflora in AS patients increased, including Lachnospiraceae, Ruminococcaceae, Rikenellaceae, Porphyromonadaceae, and Bacteroidaceae. Species

richness of Veillonellaceae and Prevotellaceae decreased [20]. However, Lin et al. [21] observed that the abundance of Prevotellaceae increased and that of Rikenellaceae

Table 4. Comparison of horizontal relative abundance of phylum level (mean \pm SD)

Group	Phylum Firmicutes	Bacteroides	Actinomycetes	Proteobacteria	Bacteroides/Firmicutes
Health group	0.77 \pm 0.14	0.10 \pm 0.11	0.06 \pm 0.08	0.06 \pm 0.12	0.15 \pm 0.17
Pretreatment group	0.75 \pm 0.16	0.15 \pm 0.17	0.07 \pm 0.11	0.03 \pm 0.03	0.26 \pm 0.37
Posttreatment group	0.80 \pm 0.11	0.15 \pm 0.13	0.02 \pm 0.03	0.02 \pm 0.02	0.22 \pm 0.22

Table 5. Comparison of relative abundance of genus level (mean \pm SD)

Group	Rare micrococcus	Roma Boots	Bifidobacterium	Streptococcus
Health group	0.14 \pm 0.14	0.04 \pm 0.08	0.04 \pm 0.06	0.02 \pm 0.03
Pretreatment group	0.23 \pm 0.16*	0.02 \pm 0.02	0.03 \pm 0.07*	0.01 \pm 0.01*
Posttreatment group	0.17 \pm 0.13	0.06 \pm 0.14 [△]	0.01 \pm 0.02	0.05 \pm 0.11

*Compared with the Health group, $p < 0.05$. [△]Compared with the Pretreatment group, $p < 0.05$.

decreased in HLA-B27 transgenic rats. Although the flora structure is influenced by many factors, there is no unified conclusion on the species of different bacteria at present, and it is certain that intestinal microflora plays a key role in the pathogenesis of AS. At present, there are many targeted intestinal flora therapies to prevent or treat AS, including using antibiotics to eliminate harmful bacteria in the intestine, taking probiotics and prebiotics or increasing beneficial bacteria through fecal microflora transplantation, and using biological agents or Chinese herbal medicines to improve intestinal microecology [22].

The results of this study show that after long-snake moxibustion treatment, the symptom score of AS patients is significantly lower than before, suggesting that long-snake moxibustion can improve the clinical symptoms of AS. *Romboutsia* has the potential to produce short-chain fatty acids (SCFAs) [23, 24], and SCFA is a beneficial metabolite in the intestine which is very important to maintain the stable balance of mammalian immune system. SCFA, including acetic acid, propionic acid, and butyric acid, is formed in the microbial fermentation process of carbohydrate from diet or host, which provides energy for the host, but also mediates the interaction with the immune system [25]. The latter can not only improve the intestinal mucosal barrier function but also regulate the intestinal energy homeostasis, enhance the intestinal immune cell function, inhibit the production of pro-inflammatory cytokines in the intestine, and promote the expression of anti-inflammatory factors, and has anti-inflammatory activity. Studies have shown that the species producing butyrate in fecal samples of inflammatory bowel disease patients decreased and the level of SCFA decreased. In addition, SCFA can also induce the proliferation and differentiation of Treg cells, and the accumulation of Treg

cells in inflammatory joints contributes to the spontaneous regression and relief of peripheral spinal arthritis, and its functional defect can promote the occurrence and development of AS [18, 22]. The above evidence shows that when the abundance and level of SCFA-producing flora in the intestine increase, it is beneficial to the prognosis of AS diseases. Our experiment found that the *Romboutsia* of intestinal flora in patients with AS showed a downward trend compared with the healthy group. After long-snake moxibustion treatment, the relative abundance of the genus was significantly higher than that before treatment, suggesting that long-snake moxibustion may promote the formation of SCFA by improving the relative abundance level of species in the intestine, thus playing a role in treating AS.

The research design is highly pragmatic, considering various real-life factors, and features rigorous data analysis and presentation of results. Therefore, in terms of generalizability to the “real-world” population, this study demonstrates significant external validity.

One of the limitations of our study is the small sample size. Although the study included 30 AS patients and 30 healthy volunteers, the sample size remains limited for the complex analysis of the microbiome. Additionally, the male-to-female ratio in Chinese AS patients is typically 2–4:1, whereas in our study, it was 1:1. This disparity may affect the reliability and generalizability of certain statistical analyses. Another shortcoming is the lack of future research directions. While the article mentions that moxibustion may improve AS symptoms by modulating the gut microbiome, it does not delve into future research directions, such as gaining a deeper understanding of treatment mechanisms or conducting larger scale clinical trials.

Conclusion

Our research shows that long-snake moxibustion improves the clinical symptoms of patients with ankylosing spondylitis by regulating the abundance of intestinal microorganisms, increasing beneficial bacteria, and maintaining the steady state of intestinal microorganisms, which provides a new theoretical basis for clinical use of long-snake moxibustion to treat ankylosing spondylitis.

Statement of Ethics

This research plan was approved by the Nanchang Hongdu Hospital of Traditional Chinese Medicine Medical Ethics Committee (KY-2022-007). Written informed consent to participate in the study has been obtained from the participants.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

References

- Pipikos T, Kassimos D, Angelidis G, Koutsikos J. Bone single photon emission/computed tomography in the detection of sacroiliitis in seronegative spondyloarthritis: a comparison with magnetic resonance imaging. *Mol Imaging Radionucl Ther*. 2017; 26(3):101–9. <https://doi.org/10.4274/mirt.50570>
- Zao A, Cantista P. The role of land and aquatic exercise in ankylosing spondylitis: a systematic review. *Rheumatol Int*. 2017;37(12):1979–90. <https://doi.org/10.1007/s00296-017-3829-8>
- Rudwaleit M, van der Heijde D, Khan MA, Braun J, Sieper J. How to diagnose axial spondyloarthritis early. *Ann Rheum Dis*. 2004;63(5):535–43. <https://doi.org/10.1136/ard.2003.011247>
- Ward MM, Deodhar A, Gensler LS, Dubreuil M, Yu D, Khan MA, et al. 2019 update of the American college of rheumatology/spondylitis association of America/spondyloarthritis research and treatment network recommendations for the treatment of ankylosing spondylitis and nonradiographic axial spondyloarthritis. *Arthritis Care Res*. 2019;71(10):1285–99. <https://doi.org/10.1002/acr.24025>
- Wang R, Ward MM. Epidemiology of axial spondyloarthritis: an update. *Curr Opin Rheumatol*. 2018;30(2):137–43. <https://doi.org/10.1097/BOR.0000000000000475>
- Wang H, Zheng H, Ma Y. Drug treatment of ankylosing spondylitis and related complications: an overlook review. *Ann Palliat Med*. 2020;9(4):2279–85. <https://doi.org/10.21037/apm-20-277>
- Yu XJ, Huang H, Hu XW. Study on the mechanism of PPAR γ activated by long snake moxibustion to improve the initial ankylosing spondylitis in rats. *J Emerg Tradit Chin Med*. 2022;31(09):1372–6.
- He J, Li M, Li C. Meta-analysis of governor vessel in the treatment of ankylosing spondylitis. *Chin Arch traditional Chin Med*. 2020;38(02):102–7.
- Zhan JP, Wang HL, Miao XY. Based on the determination of key enzymes in TCA cycle, this paper studies the curative effect and mechanism of Governor vessel in the treatment of early ankylosing spondylitis patients. *World science and technology-modernization of traditional Chinese medicine*. 2022;24(09):3548–55.
- Zhou C, Zhao H, Xiao X, Chen B, Guo RJ, Wang Q, et al. Metagenomic profiling of the pro-inflammatory gut microbiota in ankylosing spondylitis. *J Autoimmun*. 2020;107:102360. <https://doi.org/10.1016/j.jaut.2019.102360>
- Rheumatology Branch of Chinese Medical Association. Guidelines for diagnosis and treatment of ankylosing spondylitis. *Chin J Rheumatol* 2010;14(8):557–9.
- Huskisson EC. Measurement of pain. *Lancet*. 1974;2(7889):1127–31. [https://doi.org/10.1016/s0140-6736\(74\)90884-8](https://doi.org/10.1016/s0140-6736(74)90884-8)
- Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. *J Rheumatol*. 1994;21(12):2286–91.
- Scher JU, Abramson SB. The microbiome and rheumatoid arthritis. *Nat Rev Rheumatol*. 2011;7(10):569–78. <https://doi.org/10.1038/nrrheum.2011.121>
- Rodríguez JM, Murphy K, Stanton C, Ross RP, Kober, OI, Juge, N, et al. The composition of the gut microbiota throughout life, with an emphasis on early life. *Microb Ecol Health Dis*. 2015;26:26050. <https://doi.org/10.3402/mehd.v26.26050>
- Calin A, Garrett S, Whitelock H, Kennedy LG, O’Hea J, Mallorie P, et al. A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. *J Rheumatol*. 1994;21(12):2281–5.
- Kim D, Yoo SA, Kim WU. Gut microbiota in autoimmunity: potential for clinical applications. *Arch Pharm Res*. 2016;39(11):1565–76. <https://doi.org/10.1007/s12272-016-0796-7>
- Scalise G, Ciancio A, Mauro D, Ciccia F. Intestinal microbial metabolites in ankylosing spondylitis. *J Clin Med*. 2021;10(15):3354. <https://doi.org/10.3390/jcm10153354>
- Stebbing S, Munro K, Simon MA, Tannock G, Highton J, Harmsen H, et al. Comparison of the faecal microflora of patients with ankylosing spondylitis and controls using molecular methods of analysis. *Rheumatol*. 2002; 41(12):1395–401. <https://doi.org/10.1093/rheumatology/41.12.1395>
- Costello ME, Ciccia F, Willner D, Warrington N, Robinson PC, Gardiner B, et al. Brief report: intestinal dysbiosis in ankylosing spondylitis. *Arthritis Rheumatol*. 2015;67(3):686–91. <https://doi.org/10.1002/art.38967>
- Lin P, Bach M, Asquith M, Lee AY, Akileswaran L, Stauffer P, et al. HLA-B27 and human β 2-microglobulin affect the gut microbiota of transgenic rats. *PLoS One*. 2014;9(8):e105684. <https://doi.org/10.1371/journal.pone.0105684>

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Author Contributions

Y.X. and H.X. conceived the study. Y.X. and J.M. performed the study and drafted the manuscript. H.H., Q.F., G.L., G.R., and Z.X. designed the study and participated in data acquisition. All authors read and approved the final version of the manuscript accepted for publication.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

- 22 Song ZY, Yuan D, Zhang SX. Role of the microbiome and its metabolites in ankylosing spondylitis. *Front Immunol.* 2022;13:1010572. <https://doi.org/10.3389/fimmu.2022.1010572>
- 23 Zhang X, Zhang B, Peng B, Wang, J, Hu, Y, Wang, R, et al. Different dose of sucrose consumption divergently influences gut microbiota and PPAR- γ /MAPK/NF- κ B pathway in DSS-induced colitis mice. *Nutrients.* 2022;14(13):2765. <https://doi.org/10.3390/nu14132765>
- 24 Ye G, Zhang L, Wang M, Chen, Y, Gu, S, Wang, K, et al. The gut microbiota in women suffering from gestational diabetes mellitus with the failure of glycemic control by lifestyle modification. *J Diabetes Res.* 2019. 2019:6081248–12. <https://doi.org/10.1155/2019/6081248>
- 25 Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol.* 2014; 12(10):661–72. <https://doi.org/10.1038/nrmicro3344>