

Phytochemical Analysis and Susceptibility of Multi-Drug Resistant Typhoidal *Salmonella* Species to Combined Ethanolic Extract of *Boswellia Dalzielii* and *Tamarindus Indica*

ZUMBES Hosea Jwan., *KWAPNOE Aaron Peter., DAVID John., AZI Hezekiah Yusuf., EKPIWRE Veronica David

Department of Microbiology, Faculty of Natural Science, University of Jos, Jos, Nigeria

*Corresponding Author

DOI: <https://doi.org/10.51584/IJRIAS.2026.11SP140007>

Received: 14 June 2026; Accepted: 20 June 2026; Published: 07 July 2026

ABSTRACT

The world health sector has been held hostage by the recent surge in antimicrobial resistance in disease management. Confirmed multi-drug-resistant *Salmonella Typhi* and *Salmonella Paratyphi A* isolates were obtained from the National Veterinary Institute, Vom. The efficacy of combined ethanolic crude extracts of fruit pulp of *Tamarindus indica* and stem bark of *Boswellia dalzielii* was analyzed in vitro, in combined form (1:1), on the test organisms *S. Typhi* (ST) and *S. Paratyphi A* (SPTA). The plant extracts were screened for phytochemical bioactive compounds, using a qualitative approach and GC-MS for quantitative assays. *Salmonella Typhi* and *Salmonella Paratyphi* isolates were susceptible to the combined plant extracts at concentrations of 100-400 mg/ml. The inhibition zones recorded are 31 and 42mm for *S. Paratyphi A* and 26 and 38mm for *S. Typhi* for extract concentrations of 100 and 400mg/mL, respectively. Furthermore, the Minimum Inhibitory Concentration (MIC) of 3.12mg/mL was recorded for *S. Paratyphi A*, and 12.5mg/mL for *S. Typhimurium*. The plant extracts exhibited antimicrobial efficacy on the typhoidal test organisms. The GC-MS analysis of the combined ethanolic plants extract revealed the presence of 5-hydroxymethylfurfural (34.48%), 3-O-methyl-d-glucose (13.39%), Furfural (7.19%), 4H-pyran-4-One-2,3,-dihydro-3,5-dihydroxy-6-methyl- (4.82%), Furfural hydroxymethyl ketone (4.80%), Naphthalene,decahydro-4a-methyl-1-methyl-7-(1-methyl ethylidene) - (3.94%), Cis-9-Hexadecanal (3.82%), 9-Oxabicyclo[6.1.0]non-6-en-2-one (2.86%), and 2(5H)-Furanone,5-methyl- (1.68%) had the least concentration. The combined efficacy of the plant extracts confirms and underscores their synergistic effects on the test isolates, thereby making the plants a significant alternative for drug sourcing against multi-drug-resistant *Salmonella Typhi* and *Salmonella Paratyphi A*.

Keywords: Drug-resistance, Salmonella, Gastritis, Phytomedicine

INTRODUCTION

In ancient and recent times, humans developed a sense of urgency in developing treatment strategies against ailments that have plagued and caused economic loss to the human race. Hence, man has been compelled to search for better treatment strategies against disease as the causative agents of the disease have evolved and developed resistance against the developed treatment techniques [1]. The rate at which microorganisms develop mechanisms to evade the activity of antimicrobials, rendering the antimicrobials ineffective, poses a significant threat to world economies as multidrug resistance has held the health and food sectors hostage, thereby increasing morbidity and mortality rates [2,3]. The plants *Tamarindus indica*, which is called *Tamarin* in the English language and is commonly known as “*Dwang*” in the Tangle (Tangale) language, and “*Tsamiya*” in Hausa [4] and *Boswellia dalzielii*, commonly known as the frankincense tree or by its Tangle (Tangale) name “*shomnong*”, Hausa names (“*Ararrabi*”, “*Basamu*” and “*Hanu*”) [5]. Generally, plants have been used by herbalists and traditionalists in the treatment of certain disease conditions, which include wound healing, snake bite, abdominal pain, colds, inflammations, diarrhea, helminth infections, and fever treatments, among others [6].

It has been previously reported that the use of *Tamarindus indica* as a probable source of antimicrobial, antidiabetic, and anti-inflammatory, potentially treating or preventing obesity and other chronic diseases [7]. The antimicrobial activity of *T. indica* has been established on various test organisms [8]. Plants produce certain compounds that have the potential to inhibit the growth of microorganisms as well as cidal effects, and it has been observed that various and different parts of plants produce varying antimicrobial characteristics [9,10]. This signifies the importance of plants as an alternative source of safe and potent antimicrobials. Phytomedicine is highly promising in the area of drug development.

The human-restricted serovar *Typhi* (ST) and the closely related serovar *ParaTyphi* A (SPTA) cause enteric fever and cause a systemic disease [11]. Enteric fever, SPTA causes a milder disease with a shorter incubation time [12]. As earlier reported, typhoid fever has caused at least 215000 deaths as well as 26 million cases of morbidity in typhoid cases and 5 million in paratyphoid disease conditions [13]. The burden of typhoid fever is generally felt in developing and low-income countries and regions of the world, with Africa and Southeast Asia being the regions with the most risk [14]. Furthermore, invasive nontyphoidal *Salmonella* has emerged as an important cause of bloodstream infection in Sub-Saharan Africa in both adults and children. The incidence of invasive non-typhoidal *Salmonella* has been estimated at 3.4 million cases with more than 600,000 deaths each year [15]. This poses a serious global health challenge, including antibiotic resistance, and it is also a major health security issue [16]. AMR is speedily gaining global recognition and in-depth scientific discussion; hence, there is a need to proffer alternative(s) for antimicrobials from natural sources that overcome the threat of resistance and improve healthcare delivery.

MATERIALS AND METHODS

Test Isolates

The test isolates were obtained from the National Veterinary Research Institute, Vom (NVRI); the Microbiology unit of the research institute has already established its multi-drug resistance. The isolates obtained were *Salmonella Typhi* and *Salmonella ParaTyphi* A.

Susceptibility testing

The susceptibility test was conducted using the disc diffusion method [17]. Sterile discs were impregnated with the plant extract sample and placed on a cultured plate containing the test isolates, and the zones of inhibition were measured after 24 hours of incubation.

Plant Preparation

The plant sample, which includes the stem bark of *Boswellia dalzielii* and the fruits of *Tamarindus indica*, was obtained from the farm, dried at room temperature, and crushed into powder using a mortar. The crushed powder was sieved to remove large lumps of plant fibre; the fine powder was used for extraction.

Phytochemical Screening

Qualitative screening: the combined and separate plant extracts were screened for the presence of bioactive compounds; the methods [18]. Quantitative analysis: bioactive components of the extract were determined using the GC-MS approach.

RESULTS AND DISCUSSION

Susceptibility of the Test Organism to the Plant Extracts

Table 1 shows the zones of inhibition exhibited by *Tamarindus indica* and *Boswellia dalzielii* plant extracts. The highest zones of inhibition are 20mm and 18mm for *Salmonella ParaTyphi* A and *Salmonella Typhi*, respectively, at the concentration of 400mg/mL of *Tamarindus indica* extract, 18mm and 17mm for *Salmonella ParaTyphi* A and *Salmonella Typhi*, respectively, at the concentration of 400mg/mL of *Boswellia dalzielii*

extract. However, the lowest susceptibility was recorded at 200mg/mL for both plant extracts. The reported zones of inhibition in this study differ from the previously reported 7.67mm zone of inhibition at 500mg/mL for *B. dalzielii* butanol extract [10]. And, similar to the report of 19mm at 50mg/mL [9]. And, differ considerably of the 12mm zone of inhibition on *S. typhi* for *T. indica* [19]. The reported zones of inhibition vary considerably from the earlier reported zones of inhibition; this can be attributed to the varying experimental extract concentration administered.

Table 1. Susceptibility of the Test Organism to *Tamarindus indica* and *Boswellia dalzielii* Plant Extract

Extract	Tiet (mg/mL)			BDet (mg/mL)			Control (mg/mL)	
Isolate	400	200	100	400	200	100	+ve	-ve
ST	18	15	12	17	13	10	31	0
SPTA	20	17	14	18	15	11	33	0

Table 2 shows the result of the Susceptibility Test of Bacterial isolates to Combined plant extracts at the Ratio (1:1), the zones of inhibition (mm). The combined plants' extracts have exhibited activity against the bacterial isolates at 400mg/mL, with a higher zone of inhibition of 42mm for *S. Paratyphi* A and 38 mm for *S. Typhi* when tested with the combination of the plants' extracts at the ratio of 1:1. Thus, indicating the synergistic effect of the plant extracts by enhancing the antimicrobial efficacy of the plant's phytochemical components.

Table 2. Susceptibility of the Test Organism to the combined (1:1) Extracts of *Tamarindus indica* and *Boswellia dalzielii*

Extract	Tiet+BDet (mg/mL)			Control (mg/mL)	
Isolate	400	200	100	+ve	-ve
ST	38	33	26	31	0
SPTA	42	36	31	32	0

KEY: Tiet=*Tamarindus indica* ethanolic, BDet=*Boswellia dalzielii* ethanolic, ST=*Salmonella Typhi*, SPT A=*Salmonella ParaTyphi* A, Ext. = Extract, Positive = +, Negative = -.

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Tamarindus indica* and *Boswellia dalzielii* Plant Extract

Table 3 shows the MIC and MBC of the individual plants' extracts. 12.5mg/mL and 6.25mg/mL were the MICs for *Salmonella Paratyphi* A and *Salmonella Typhi*, respectively, for *Tamarindus indica* ethanolic extract; these were the same for *Boswellia dalzielii*. However, 12.5mg/mL and 25.0mg/mL were the MBC for *Salmonella Paratyphi* A and *Salmonella Typhi*, respectively, for *Tamarindus indica* extract. However, the MBC for *Salmonella Paratyphi* A for *Boswellia dalzielii* extract varied and was recorded at 25.0mg/mL. The reported MIC and MBC of this study differ from the findings of Balarabe *et al.* [10], who reported the antimicrobial activity of *Boswellia dalzeili* on *S. Typhi*, reporting an MIC of 7.81 and MBC of 62.50 in methanol extracts; this contrasts with the 12.5 (MIC) and 25.0 (MBC) reported in this study. However, the reported MIC and MBC are similarly reported as 12.5 and 25, respectively [9]. However, for *T. indica*, contrastingly, the MIC and MBC are 125 and 125, respectively, for *S. typhi* [19].

Table 3. MIC and MBC of *Tamarindus indica* and *Boswellia dalzielii* Plant Extracts on the Test Organism

Extract	Tiet (mg/mL)		BDet (mg/mL)		Control (mg/mL)	
Isolate	MIC	MBC	MIC	MBC	MIC	MBC
ST	12.5	25.0	12.5	25.0	0.04	0.08
SPTA	6.25	12.5	6.25	25.0	0.04	0.16

Table 4 shows the result of the MIC and MBC of the combined plant extracts at a ratio of 1:1. The MIC and MBC of the combined *Tamarindus indica* and *Boswellia dalzielii* plant extract, 3.12 mg/ml, was the lowest

MIC obtained against *S. ParaTyphi* A, while the highest MIC of 6.25 mg/ml was obtained for *S. Typhi*. The lowest MBC of 6.25 mg/ml was obtained against *S. ParaTyphi* A, while the highest MBC of 12.5 mg/ml was obtained for *S. Typhi*. By exhibiting lower MIC and MBC, *S. ParaTyphi* A is the most susceptible to the extracts. The combined plant extracts exhibit lower MIC and MBC; thus, highlighting the efficacy and potency of the plants extract to be a good and safe alternative source antimicrobial.

Table 4. MIC and MBC of the combined *Tamarindus indica* and *Boswellia dalzielii* Plant Extracts at the Ratio of 1:1 on the Test organism

Extract	Tlet+BDet (mg/mL)		Control (mg/mL)	
Isolate	MIC	MBC	MIC	MBC
ST	6.25	12.50	0.04	0.08
SPTA	3.12	6.25	0.04	0.16

KEY: Tlet=*Tamarindus indica* ethanolic, BDet=*Boswellia dalzielii* ethanolic, ST=*Salmonella Typhi*, SPTA=*Salmonella ParaTyphi* A, Ext. = Extract.

Qualitative Phytochemical Screening of the Plant Extract

Table 5, qualitative phytochemical constituents of the ethanolic extracts of *Boswellia dalzielii* and *Tamarindus indica*, separately and in combined ratios (1:1). Based on phytochemical test results, various bioactive compounds (secondary metabolites) that might be responsible for the medicinal attributes of the plants (medicinal property) were identified. Tannins were present in the plant extracts analyzed. Flavonoids were present in the plant extracts. Saponins were present except for the *Tamarindus indica* ethanolic extract. Steroids were present; additionally, Cardiac Glycosides were present. However, Alkaloids were absent in both plant extracts.

Table 5. Qualitative Phytochemical Constituents of the Plant Extracts

Combined Metabolites	BDet	Tlet	extract
Tannins	+	+	+
Saponins	+	-	+
Steroids	+	+	+
Cardiac glycoside	+	+	+
Alkaloid	-	-	-
Flavonoids	+	+	+

KEY: BDet=*Boswellia dalzielii* ethanolic, Tlet=*Tamarindus indica* ethanolic, Present = +, absent = -

The preliminary phytochemical (qualitative) screening results show the outcome for the combined ethanolic extracts: Stem bark of *B. dalzielii* and fruit pulp of *T. indica*. Based on phytochemical tests, secondary metabolites are responsible for the antimicrobial attributes of the plants [9]. These findings are similar to the earlier reported presence of tannins, glycosides, flavonoids, and steroids in *T. indica* extract [20]. In the same vein, reports the presence of saponin, tannin, flavonoids, cardiac glycosides, steroids, terpenes and phenol in ethanol extracts, and alkaloids were absent [21,22]. Furthermore, the presence of flavonoids that are well-known as strong antioxidants makes *T. indica* leaves a promising natural antioxidant source [23].

Quantitative Phytochemical Constituents of the Combined Ethanolic Plant Extracts

Table 6, below, shows the bioactive components identified with major Peaks from the Combined Ethanolic Extracts of *B. dalzielii* and *T. indica* (1:1) by GC-MS Analysis. The GCMS analysis of the combined Ethanolic extract is said to have revealed the presence of compounds; 5-hydroxymethylfurfural (34.48%) was observed to be the most predominant compound, this is followed by 3-O-methyl-d-glucose (13.39%), Furfural (7.19%), 4H-pyran-4-One-2,3,-dihydro-3,5-dihydroxy-6-methyl- (4.82%), Furfural hydroxymethyl ketone (4.80%),

Naphthalene,decahydro-4a-methyl-1-methyl-7-(1-methyl ethylidene) - (3.94%), Cis-9-Hexadecanal (3.82%), 9-Oxabicyclo[6.1.0]non-6-en-2-one (2.86%), and 2(5H)-Furanone,5-methyl- (1.68%) had the least concentration. Earlier reports found the presence of 5-Hydroxymethylfurfural (31.06%) and 3-O-Methyl-d-glucose (16.31%) in *T. indica* extracts [24]. Most importantly, the pathogenicity-mitigating capability of 5-Hydroxymethylfurfural by suppressing the quorum-sensing phenotype and biofilm production has been reported [25].

Table 6. Bioactive Components Identified with Major Peaks from the Combined Ethanolic Extracts of *B. dalzielii* and *T. indica* (1:1) by GC-MS Analysis

Peak No.	Retention Time (Min)	Peak Area (%)	Name
15	8.967	34.48	5-Hydroxymethylfurfural
19	13.916	13.39	3-O-Methyl-d-glucose
2	4.195	7.19	Furfural (Artificial Ant oil)
14	7.821	4.82	4H-Pyran-4-one, 2, 4-dihydro-3,5-dihydroxy- 6-methyl
11	7.013	4.80	Furylhydroxymethyl Ketone
28	19.84	3.94	Naphthalene,decahydro-4a-methyl-1-methylene-7-(1-methylidene)-
23	16.196	3.82	Cis-9-Hexanal,(Z)-
21	14.896	2.86	9-Oxabicyclo [6.1.0] non-6-en-2-one
5	5.565	1.68	2(5H)-Furanone, 5-methyl-

CONCLUSIONS

The two plant extracts exhibited strong antimicrobial activity against the test organism when used separately. However, a synergistic effect was achieved when the plant extracts were combined. The strong antimicrobial activity demonstrated by the combined plant extracts suggests that the plants can be exploited for use in the treatment of ailments such as typhoid and gastroenteritis caused by the test organisms.

Conflict of Interest

There is no conflict of interest.

REFERENCE

1. Amir, M., Khan, M. A., Ahmad, S., Akhtar, M., Mujeeb, M., Ahmad, A., Khan, S. A., and Al-Abbasi, F. A. (2016). Ameliorating effects of Tamarindus indica fruit extract on anti-tubercular drugs induced liver toxicity in rats. Natural Product Research, 30(06): 715-719.
2. Schwarz, S., Loeffler, A. and Kadlec, K. (2016). Bacterial resistance to antimicrobial agents and its impact on veterinary and human medicine. Veterinary Dermatology.
3. Oyedum, M. U., Kuta, F. A., and Garba, S. A. (2016). Retrospective Study of Multidrug-Resistant Salmonella Typhi in Nigeria. A Review Seminar was presented to the Department of Microbiology, School of Life Sciences, Federal University of Technology, Minna. Umaru Musa Yar'adua University Journal of Microbiology Research, 1(1):77-85.
4. Garba, K. G. and Hauwa, Y. (2017). Tamarindus indica: An Appraisal on Its Uses in Traditional Medicine and Its Antinutritional Components. Chronicles of Pharmaceutical Science, 1:199-203.
5. Aliyu, R., Gatsing, D. and Jaryum, K. H. (2007). The Effects of Boswellia dalzielii (Burseraceae) Aqueous Bark Extract on Rat Liver Function. Asian Journal of Biochemistry, 2: 359-363. DOI: 10.3923/ajb.2007.359.363
6. Mamza, U.T., Rabi, M. B., Khan, S., Shah, M. A., Arshad, M., Yakubu, M. I., Dawa, S., Sule, M. and Khan, I. Z. (2022). Phytochemistry and gas chromatography-mass spectrometry (GC-MS) analysis of bioactive components of methanolic root-bark extract of Boswellia dalzielii (Burseraceae). Bulletin of

- Pure & Applied Sciences. Sec. C, Physical Sciences, 41c(1):9-21. doi: 10.5958/2320-320x.2022.00002.4
7. Ribeiro, J. A., Serguiz, A. C., Silva, P. F., Barbosa, P. B., Sampaio, T. B., Araújo Junior, R. F., Olivera, A. S., Machado, R. J. A., Maciel, B., Uchôa, A. F., Santos, E. A., and Morais, A. (2015). Trypsin inhibitor from *Tamarindus indica* L. seeds reduces weight gain and food consumption and increases plasmatic cholecystokinin levels. *Clinics (Sao Paulo)*, 70(2):136-143.
8. Abukakar, M. G., Ukwuani, A. N., and Shehu, R. A., (2008). Phytochemical Screening and Antibacterial Activity of *Tamarindus Indica* Pulp Extract. *Asian Journal of Biochemistry*, 3: 134-138. DOI: [10.3923/ajb.2008.134.138](https://doi.org/10.3923/ajb.2008.134.138)
9. Salisu, B., Muhammad, A., Hussain, A., and Adamu, U. (2017). Determination of Phytochemicals and Antimicrobial Activity of Aqueous Stem Bark Extract of *Boswellia dalzielii* against Some Common Pathogenic Microorganisms. *UMYU Journal of Microbiology Research (UJMR)*. 2. 238-246. 10.47430/ujmr.1721.035.
10. Balarabe, S., Aliyu, B. S., Muhammad, H., and Nafisa, M. A. (2023). Phytochemicals And Antimicrobial Activity of *Boswellia Dalzielii* Stem Bark Against Some Clinical Isolates. *FUDMA Journal of Sciences*, 3(3):587-593.
11. Crump, J. A., and Mintz, E. D. (2010). Global trends in typhoid and paratyphoid fever. *Clinical Infectious Diseases*, 50(2), 241–246. <https://doi.org/10.1086/649541> PMID: 20014951
12. [Bhan, M. K., Bahl, R. B., and Bhatnagar, S. (2005). S. Typhoid and paratyphoid fever. *Lancet*, 366 (9487):749–762. [https://doi.org/10.1016/S0140-6736\(05\)67181-4](https://doi.org/10.1016/S0140-6736(05)67181-4) PMID: 16125594
13. Michael, C. J., and Eric, D. M. (2017). National Center for Emerging and Zoonotic Infectious Diseases (NCEZID) Division of Global Migration and Quarantine (DGMQ). CDC.
14. Dutta, S., Das, S., Mitra, U., Jain, P., Roy, I., Ganguly, S. S., Ray, U., Dutta, P., and Paul, D. K. (2014). Antimicrobial resistance, virulence profiles and molecular subtypes of *Salmonella enterica* serovars Typhi and ParaTyphi A blood isolates from Kolkata, India during 2009-2013. *PloS one*, 9(8):e101347.
15. Ao, T. T., Feasey, N. A., Gordon, M. A., Keddy, K.H., Angulo, F. J., and Crump, J. A. (2015). Global burden of invasive nontyphoidal *Salmonella* disease, 2010(1). *Emerging Infectious Diseases*, 21(6).
16. Akinyemi, K. O., Oyefolu, A. O. B., Mutiu, W. B., Iwalokun, B. A., Ayeni, E. S., Ajose, S. O. and Obaro, S. K. (2018). Typhoid Fever: Tracking the Trend in Nigeria. *American Journal of Tropical Medicine and Hygiene*, 99(3):41-47. doi:10.4269/ajtmh. 18-0045.
17. Bayot, M. L. and Bragg, B. N. (2024). Antimicrobial Susceptibility Testing. [Updated 2024 May 27]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK539714/>
18. Junaid, R. S. and Patil, M. K. (2022). Qualitative tests for preliminary phytochemical screening: An overview. *Int J Chem Stud.*, 8(2):603-608. DOI: 10.22271/chemi.2020.v8.i2i.8834
19. Nwodo, U. U., Obiiyeke, G. E., Chigor, V. N., and Okoh, A. I. (2011). Assessment of *Tamarindus indica* extracts for antibacterial activity. *International journal of molecular sciences*, 12(10), 6385–6396. <https://doi.org/10.3390/ijms12106385>
20. Akram, M., Ishaku, G., Abaka, A., Islam, Md., and Saeed, M. (2021). Phyto-pharmacology of *Tamarindus indica*. *Ciencia e Investigación Medico Estudiantil Latinoamericana*. 24. 33-40. 10.15381/ci.v24i2.22524.
21. Yakubu, J., Maina, C. A., Abdulrahman, F. I., Sodipo, O. A., Andema, K. A., and Dawi, H. A. (2022). Phytochemical Analysis, Nutritional Content and Evaluation of Antidiabetic Related Elements of *Boswellia Dalzielii*. *Nig Annals of Pure & Appl Sci.*, 5(1):97-108. DOI:10.5281/zenodo.7130093
22. Ogbu, I., Chukwudi, I., Ijomanta, O., Agwu, E., Chinonye, C., and Kespo, K. (2019). In vitro Antimicrobial and Antifungal Efficacy of Ethanol Crude Stem Bark Extract of *Boswellia dalzielii*. *Journal of Advances in Medicine and Medical Research*. 1-8. 10.9734/jammr/2019/v29i630093.
23. Sookying, S., Duangjai, A., Saokaew, S., and Phisalprapa, P. (2022). Botanical aspects, phytochemicals, and toxicity of *Tamarindus indica* leaf and a systematic review of antioxidant capacities of *T. Indica* leaf extracts. *Frontiers in Nutrition*, 9, 977015. <https://doi.org/10.3389/fnut.2022.977015>
24. Fagbemi, K. O., Aina, D. A., Adeoye-Isijola, M. O., Naidoo, K. K., Cooposamy, R. M., and Olajuyigbe, O. O. (2022). Bioactive compounds, antibacterial and antioxidant activities of the methanol extract of *Tamarindus indica* Linn. *Scientific reports*, 12(1), 9432. <https://doi.org/10.1038/s41598-022-13716-x>

-
25. Rajkumari, J., Borkotoky, S., Reddy, D., Mohanty, S. K., Kumavath, R., Murali, A., Suchiang, K., and Busi, S. (2019). Anti-quorum-sensing and anti-biofilm activity of 5-hydroxymethylfurfural against *Pseudomonas aeruginosa* PAO1: Insights from in vitro, in vivo and in silico studies. *Microbiological research*, 226, 19–26. <https://doi.org/10.1016/j.micres.2019.05.001>