

The Effectiveness of Probiotics Defending Against Antibiotic Adverse Effects

Huize Zhang^{1*}

¹ Culver Academies, Culver, Indiana, United States

Email Address

zhz3327490372@gmail.com (Huize Zhang)

*Correspondence: zhz3327490372@gmail.com

Received: 16 October 2024; **Accepted:** 17 November 2024; **Published:** 26 December 2024

Abstract:

Antibiotic abuse can cause antibiotic-associated diarrhea (AAD), collection of moderate to severe diarrheal symptoms due to disrupted intestinal environment, which may be treated by probiotics. This paper generalizes antibiotics and factors causing AAD, then enumerates available probiotic sources and mechanisms of action to provide practical suggestions to treat AAD. This research aims to be a good theoretical reference regarding to antibiotic regulations and methods to prevent AAD, contributing to promote general understanding of antibiotic usage for the public and to treat other similar antibiotic adverse effects.

Keywords:

Probiotics, Antibiotics, Antibiotic-associated Diarrhea (AAD)

1. Introduction

Prior to the invention of antibiotics, common treatment against bacterial infection included allopath, herbs, and homeopath. However, these treatments were not effective for severe bacterial infection due to surgery or accidents. Antibiotics, proven by patients' low death rate, thus became the first choice of suppressing bacterial infection and accompanying symptoms such as high fever and abscess. However, adverse effects exist when using antibiotics. Common adverse effects include nausea, headache, and antibiotic drug-resistance. Especially, unconventional symptoms after taking antibiotics such as diarrhea and watery stool, collectively called antibiotic-associated diarrhea (AAD), has about 20% morbidity among children or patients with weak gastrointestinal (GI) system.

Probiotics has recently been a field of interest for researchers to moderate AAD symptoms. However, though probiotics appear frequently in daily life as food additives, few have recognized its significance on antibiotic infection. This paper will summarize antibiotic functions and its side effects that causes AAD, then providing available probiotic source as well as general suggestions to treat the disease. This paper will provide theoretical reference to the field of antibiotic infection, explaining in details how antibiotics cause AAD. In reality, it will enrich future research on probiotic usage in AAD treatments and lower AAD morbidity.

2. Concept and Application of Antibiotics

2.1. Definition and mechanism of antibiotics

Antibiotics are substances produced by microorganisms, which can help defend against bacterial infection. After the first discovery of penicillin by Alexander Fleming during the early 20th century, antibiotics have been applied widely in medical treatments such as preventing MRSA after surgery [1]. Antibiotics has two major mechanisms to treat bacterial infection. On one hand, antibiotics inhibits bacterial DNA replication, requiring compounds such as quinolone to prevent bacterial DNA polymerase and topoisomerase from strand rejoining. After forming the quinolone-topoisomerase-DNA complex formation, the replication fork for DNA synthesis is arrested, which leads to bacteriostasis and eventually cell death. Though DNA stress response (SOS response) may be activated, preventing this response will enhance the efficacy of quinolone drugs. [1] On the other hand, antibiotics inhibits bacterial cell wall synthesis. It requires proteins to break bacterial cell wall linkage that causes cytoplasm leakage and lytic cell death. β -lactams (including cephalosporins and penicillin) and glycopeptides are two common antibiotics that interrupt specific steps during cell-wall synthesis. Specifically, β -lactams serves as a substrate to disable enzymes from forming peptidoglycan cross-links units, while glycopeptides serve as steric inhibitors of peptidoglycan maturation by blocking transpeptidase activity. Both drugs have been proved to be effective against Gram-positive bacteria due to peptidoglycan exposure. [1] Under either mechanism, antibiotics needs 2-3 days to eliminate sufficient bacteria to alleviate common symptoms.

2.2. side effects of antibiotics

Though being beneficial to fight against most bacterial infection, risks exist. “Antimicrobial resistance” often occurs after limiting patients to a single type of antibiotics for an extended period of time, which bacteria then develops genetic resistance to those antibiotics through horizontal gene transfer. Once those mutated bacteria survived successfully from single-type-antibiotic treatments, they proliferate at a high rate, rendering the same antibiotic treatment useless. Cocktail treatment may be a good alternative to prevent antimicrobial resistance in the short term, but it does not apply well in the long term [3]. Antibiotics, either used orally or intravenously, also damages organs in the digestive system or urinal system. For example, amoxicillin may partially damage renal function due to its metabolism pathway, causing oliguria and, rarely, acute interstitial nephritis(AIN) [2]. Common antibiotic side effects such as rash and dizziness may persist throughout the treatment, but will gradually disappear once stopped taking antibiotics. However, when these symptoms appear uncontrollable, considering the possibility of antibiotic-associated illness may be necessary.

3. Causes, Mechanisms, and Morbidity of AAD

3.1. causes of AAD

Clostridioides difficile, more commonly known as C.diff, is a Gram-positive, intestinal anaerobic bacteria which causes 10-20% of AAD infections. It is transmitted from person or animal to person by the fecal-oral route, and its spores can survive consistently in harsh environment [4]. They germinate and multiply into vegetative cells in the colon upon exposure to bile acids. Once spores are ingested,

their acid resistance allows them to pass through the stomach unharmed. *C.diff* is a poor competitor and is often outcompeted for nutrients by other bacteria in a normal intestinal environment, thus maintaining a manageable number. However, in a disturbed intestinal environment, *C.diff* population outgrows and causes AAD infection. Its symptom includes frequent loose, watery stool without complication, and sometimes blood in diarrhea. It is often accompanied by dehydration and an ineffective short-fatty-acid absorption [5,6].

3.2. Mechanisms of AAD

Among watery stool and uncontrollable diarrhea, the major result of AAD is the imbalance bacterial colony in the GI tract because many opportunistic pathogen and beneficial bacteria are also killed by antibiotics, along with infectious bacteria. *C. diff* colonies thus grows rapidly, damaging the GI tract. It produces up to three types of toxins: enterotoxin A, cytotoxin B, and *Clostridioides difficile* transferase [7]. Enterotoxin A helps highly-virulent toxigenic strain of *C.diff* to adhere better to the gut and cause fluid accumulation in the lumen; cytotoxin B acts synergistically with enterotoxin A to inactivate GTPase as well as to depolymerize actin in the apical membrane, leading to sodium/chloride ion movement into intestinal lumen and thus liquid flowing into the lumen due to low osmotic pressure [8]; *Clostridium difficile* transferase(CDT), which also depolymerises intestinal actin, alternates “capture proteins” which solidate the structure of microtubule, thus causing microtubule-based protrusions on the cytoskeleton. This cytoskeleton’s rupture (CDT-induced protrusions) is enlarged by septin located at the bottom of the protrusions. These processes increase pathogen adherence to intestinal cells, disrupting intestinal equilibrium [7].

3.3. Morbidity of AAD

The morbidity of AAD varies with the type of antibiotics and patient’s age. Antibiotics such as clindamycin, fluoroquinolones and cephalosporins are directly associated with AAD infections in hospital, while common antibiotics, including amoxicillin, cephalosporins, and azithromycin, also induces AAD, though within less than 1% of possibility [9]. After using antibiotics for 72 hours, around 20% of the patients will develop AAD, but usually will recover from it once stop taking antibiotics or change to other antibiotics within 8 weeks [6]. Among those patients, around 11-21% of them are children(<14 years old) while for old people (>65 years old) is 10-37% [8]. Up to 67% of neonates delivered in hospitals may be colonized with *C. difficile*, but rarely show diarrheal symptoms. The incidence among adults varies from 5% to 70%.

4. The Mechanisms of Treating AAD

One effective treatment against AAD is Faecal Microbiota Transplant(FMT) which transfers fecal microbe from a healthy person to the patient. It is proven to be successful for more than 90% of the AAD infections [10]. Besides, probiotics have been proved to largely alleviate AAD symptoms in many randomized trials [13].

4.1. Available sources of probiotics

Probiotics are living organisms that enhance health after being consumed. Two main sources of probiotics are fermented vegetables and dairy products. On one hand, fermented vegetable preserves well with low cost, energy requirements, and

improvement in nutrition [11]. Some of the most common fermented vegetables are Kimchi, Soidon, and Gundruck, where the microorganisms are from the *Lactobacillus* family, including *L. plantarum* and *L. pentosus*. On the other hand, dairy products contain several proteins, minerals, and vitamins, which are essential to nutrition and intestinal protection. Yogurt and cheese have been proved to be beneficial for preventing metabolic disorder and supporting integral physical development. Common bacteria include *Bulgaricus*, *Streptococcus thermophilus*, and other bifidobacteria [12].

4.2. Treating AAD through competitive exclusion

AAD can be prevented by competitive exclusion between intestinal bacteria and other invasive bacteria. Some probiotic bacteria such as lactobacilli adhere to the intestinal epithelium to prevent infectious bacteria from binding to the host receptors on the epithelium. Therefore, as long as there are intestinal bacteria which can bind to the receptors before other bacteria do, nutrients are preserved, thereby protecting the intestinal equilibrium from toxins or cytoskeleton protrusions. For example, enteropathogenic *E. coli* are known to bind to epithelial cells via mannose receptors [14]. Also, the group of partial antibody blocking of Toll-like receptor (TLR) by SIEC can increase the inhibitory effect twice as much as the control group (F9). Some adhesive probiotic bacteria, such as *L. plantarum* 299v, *L. acidophilus* ATCC4356, and *Streptococcus thermophilus* ATCC19258 have been shown to prevent pathogen-induced electrolyte secretion and barrier dysfunction [14]. These probiotic bacteria protect host receptors from potential pathogen infections.

4.3. Enhancement of epithelial barrier

AAD can be alleviated through enhancement of epithelial barrier function. Bacteria may enter the intestinal tight junctions by secreting toxins to break down cytoskeleton. Commensal bacteria and probiotics thus produce proteins which help seal the tight junction and tight apical intestinal tissues, limiting the entry of invasive bacteria. Some probiotic bacteria can up-regulate mucin production from goblet cells to maintain cytoskeleton and mucosal generation. For example, when *Lactobacillus casei* CGMCC 12435 and a mix of *Lactobacillus* and *Bifidobacterium* strains are given to mice with ampicillin treatment, the results showed that there were increases in tight junction proteins transcripts. [14] Especially, *L. plantarum*, a Gram-positive, non-pathogenic bacterium living in the human saliva and GI tract, has been proved in mice its successful adhesion to intestinal epithelium and strong aggregation with either other *L. plantarum* or *E. coli*, which proves their enhancement on epithelium [15]. Probiotic bacteria therefore prevent infectious bacteria from disrupting intestinal environment by strengthening tight junctions.

4.4. Modification of local environment

AAD can be relieved through modification of local environment. Without probiotics, water enters the lumen through aquaporin due to high osmotic pressure in the lumen with the existence of pathogen, undigested CHO, deficiency in short-chain-fatty-acid(SCFA), etc [16]. In contrast, with probiotics, the osmogradients is recovered by increasing amount of SCFA which promotes water and NaCl reabsorption between lumen and apical membrane. Probiotics also lowers luminal pH and oxygen levels as well as provides substrates used for other bacterial GI inhabitants. For example, in a randomized, double-blind, placebo-controlled trial, the result of using *L. johnsonii*

LA1 to healthy volunteers could increase total numbers of bifidobacteria and lactobacilli, as well as fecal lactic acid concentrations, which implies a decreased fecal pH and reduced numbers of clostridia like *C.difficile* [14]. Similarly, the presence of Lactobacilli help disform lipid micelle that traps cholesterol by producing Conjugate Bile acid Hydrolases(CBH), thereby alleviating hyperlipidemia as well as obesity [16]. By changing the nutrient composition in the intestine, probiotic bacteria can alleviate diarrhea symptoms due to AAD.

4.5. Enactment of host immune system

AAD can be moderated by enacting the host immune system, thus suppressing intestinal inflammation. Commensal bacteria mediate signals to immunosensory cells, then to T cells to eliminate the pathogens. Dendritic cells(DC) and cytokines are the two components that cooperated the most in immune responses. By reducing T cells' and cytokine's participation in mucus, intestinal inflammatory can be attenuated. For example, compared with patients receiving placebo treatment(93%), patients receiving probiotics containing *Lactobacillus reuteri* demonstrated lower levels of pro-inflammatory cytokines and NF- κ B and higher levels of interleukin-10 at one year(20%) [14]. IgG is positively regulated by antigenic stimulation in many mucosal sites (mainly nasal, tracheal, and intestinal) and has been discovered to be protective particularly for urogenital infections [14]. Maintaining the balance between immune response against pathogen and the intestinal inflammation is how probiotic bacteria enact host immune system to reduce AAD symptoms.

5. Suggestions and Improvements Regarding to Probiotics Treatment and Diagnosing AAD

Combined with previous researches, most of the patients have their symptoms alleviated, proving probiotics' necessity during AAD treatment. However, no single test can accurately prove AAD's presence. Also, the disadvantages of probiotics are not ignorable, including intestinal environment compatibility and endurance against changeable temperature and pH level [18].

5.1. diversify testings and treatments for AAD

While *C.difficile* is often referred to the indicator of AAD, there is no single test that can detect precisely the presence of *C.difficile* in stool sample. For example, while Toxin Enzyme Immunoassay (Toxin EIA) has high specificity towards *C.difficile* infection (mostly 90% and above), it is not sensitive enough (mostly 50%); Nucleic Acid Amplification Test (NAAT), on the other hand, has higher sensitivity, but it is time-consuming and often inaccurate (50% of accuracy) [19]. Therefore, If appropriate, patients could ask doctors if they could do more tests to confirm the possibility of having AAD. Patients should also be aware that *C.difficile*'s presence in intestine is not readily equivalent to AAD diagnosis since sometimes typical symptoms and toxins of AAD are not detected. Besides, no single treatment can cure AAD completely. Though probiotics can alleviate AAD symptoms, they are usually not prescribed together with antibiotics at first, except that patients have mentioned abnormality when reexamining with doctors. Similarly, after FMT, faecal recovery from *Lactobacillus rhamnosus* GG (LGG) that causes probiotic-related sepsis does not represent an optimal outcome since other determinant factors, such as gastric pH and bacterial colony composition, exist [17].

5.2. adjusting the time difference between taking antibiotics and probiotics

Some adhesive probiotic bacteria are able to prevent abnormal electrolyte production due to bacterial infection, but these protective effects were only observed when the probiotics were added before or after taking antibiotics for a certain time. For example, regarding to Probiotics such as Compound Eosinophil-Lactobacillus Tablets and Bacillus Licheniformis Capsule, patients could only take them at least 3 hours after antibiotics are taken [21,23]. Therefore, depending on the types of probiotics, there are regulations regarding to the time when probiotics should be taken. In most probiotic supplement, to ensure probiotic efficacy, patients should follow the prescription strictly. Since antibiotics are specific to bacteria (including probiotics), the beneficial effects are often offset if taken together. Recommended cycle of taking probiotics is 1-4 weeks, and may extend to 8 weeks depending on the severity of intestinal environment [21]. Taking random probiotics without being aware of the time difference may worsen AAD symptoms, because Some species in the Lactobacillus family degrades intestinal mucin, which may lead to further bacterial infection without that protective barrier on intestines.

5.3. improve probiotics survival rate

Probiotics frequently lose viability due to thermal change, drying process by denaturing its protein or osmotic pressure, so at least 10^6 CFU/ml probiotic bacteria can ensure an adequate survival rate after ingestion to treat intestinal diseases [18]. For fermented vegetables, adding salt induces plasmolysis in plant cells, creating a liquid phase on the vegetables to prevent oxidation and to reduce the amount of nitrite detrimental to health. For dairy products, adequate packaging and storage are the two most common methods to protect probiotic bacteria. By changing thin, plastic packaging to thick, glass packaging for yogurts, probiotic viability increased due to lower oxygen content. Storing strategies such as the Spray-freezing drying technique through which probiotic liquid is frozen in liquid nitrogen can reach an optimal probiotic survival rate of 97.7% [24]. Viability loss may also due to additives. For example. sugar should not exceed 10% of that of yogurt, otherwise will bring potential inhibitory effects on the probiotic bacteria colony [24]. Therefore, buying yogurts with less additives (such as Greek Yogurts) will ensure a maximum survival rate for probiotics.

6. Conclusions

Probiotics treats well a collection of diarrheal symptoms called AAD caused by antibiotic abuse. Antibiotics eliminates most bacterial infections by hindering DNA replication and breaking down peptidoglycan cell wall, but may develop antimicrobial resistance and disrupt intestinal homeostasis. When the population of bacteria *C.difficile* outcompetes other beneficial bacteria in the intestine, it leads to uncontrollable diarrheal symptoms collectively called AAD. Though the morbidity varies (5%-70%), treatments such as FMT and probiotics have been proved effective. Common probiotic sources include fermented vegetables and dairy products, which alleviates AAD by competitively excluding infectious bacteria, enhancing intestinal barrier, modifying local environment, and triggering immune response, thus increasing the absorption of certain nutrients. Despite of the benefits, patients should still be aware of potential risks: a single AAD testing may not accurately indicate presence of AAD, so patients should actively ask doctors to diversify testing types; because of its mechanism of action, antibiotics may accidentally eliminate probiotics, so

patients should consider the time difference of taking both medicines; Effective packaging, storage, and less additives can increase survival rate for probiotic bacteria, so customers should read the ingredient lists carefully when purchasing fermented vegetables or dairy products.

Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this article.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

References

- [1] Kohanski, M.A.; Dwyer, D.J.; Collins, J.J. How Antibiotics Kill bacteria: from Targets to Networks. *Nature Reviews Microbiology*, 2010, 8(6), 423-435. DOI: <https://doi.org/10.1038/nrmicro2333>.
- [2] *Amoxicillin: uses, interactions, mechanism of action* | DrugBank Online. (n.d.). DrugBank. Available online: <https://go.drugbank.com/drugs/DB01060m> (accessed on 8 July 2024).
- [3] Habboush, Y.; Guzman, N. *Antibiotic resistance*. StatPearls - NCBI Bookshelf. Available online: <https://www.ncbi.nlm.nih.gov/books/NBK513277/> (accessed on 8 July 2024).
- [4] McFarland, L.V.; Ozen, M.; Dinleyici, E.C.; Goh, S. Comparison of pediatric and adult antibiotic-associated diarrhea and *Clostridium difficile* infections. *World Journal of Gastroenterology*, 2016, 22(11), 3078. DOI: <https://doi.org/10.3748/wjg.v22.i11.3078>.
- [5] Bignardi, G. Risk factors for *Clostridium difficile* infection. *Journal of Hospital Infection*, 1998, 40(1), 1-15. DOI: [https://doi.org/10.1016/s0195-6701\(98\)90019-6](https://doi.org/10.1016/s0195-6701(98)90019-6).
- [6] Harvard Health. *Antibiotic-associated diarrhea*. Available online: https://www.health.harvard.edu/a_to_z/antibiotic-associated-diarrhea-a-to-z (accessed on 8 July 2024).
- [7] Nölke, T.; Schwan, C.; Lehmann, F.; Østevold, K.; Pertz, O.; Aktories, K. Septins guide microtubule protrusions induced by actin-depolymerizing toxins like *Clostridium difficile* transferase (CDT). *Proceedings of the National Academy of Sciences*, 2016, 113(28), 7870-7875. DOI: <https://doi.org/10.1073/pnas.1522717113>.
- [8] Zhou, H.; Xu, Q.; Liu, Y.; Guo, L.T. Risk factors, incidence, and morbidity associated with antibiotic-associated diarrhea in intensive care unit patients receiving antibiotic monotherapy. *World Journal of Clinical Cases*, 2020, 8(10), 1908-1915. DOI: <https://doi.org/10.12998/wjcc.v8.i10.1908>.
- [9] Abdur, R.; Jahan, S.; Farooq, U.; Akhtar, F.; Irshad, M.; Summiya Nizamuddin.; Parveen, A. Antibiotics Associated With *Clostridium difficile* Infection. *Cureus*, 2023, 15(5). DOI: <https://doi.org/10.7759/cureus.39029>.

- [10] Kim, K.O.; Gluck, M. Fecal Microbiota Transplantation: An Update on Clinical Practice. *Clinical Endoscopy*, 2019, 52(2), 137-143. DOI: <https://doi.org/10.5946/ce.2019.009>.
- [11] Swain, M.R.; Anandharaj, M.; Ray, R.C.; Parveen Rani, R. Fermented Fruits and Vegetables of Asia: A Potential Source of Probiotics. *Biotechnology Research International*, 2014, 1-19. DOI: <https://doi.org/10.1155/2014/250424>.
- [12] *Descriptive food science*. (n.d.). Google Books. Available online: https://books.google.com.sg/books?hl=zh-CN&lr=&id=ni-RDwAAQBAJ&oi=fnd&pg=PA3&dq=dairy+production+process&ots=oAq6lvWtAE&sig=2tixxYH8aKTyJQL_z_LO2UgsWks&redir_esc=y#v=onepage&q=dairy%20production%20process&f=false (accessed on 10 July 2024).
- [13] Newberry, S.J. Probiotics for the prevention and treatment of Antibiotic-Associated diarrhea. *JAMA*, 2012, 307(18), 1959. DOI: <https://doi.org/10.1001/jama.2012.3507>.
- [14] O'Hara, A.M.; Shanahan, F. Mechanisms of action of probiotics in intestinal diseases. *The Scientific World JOURNAL*, 2012, 7, 31-46. DOI: <https://doi.org/10.1100/tsw.2007.26>.
- [15] Lapsiri, W.; Nitisinprasert, S.; Wanchaitanawong, P. Lactobacillus plantarum Strains from Fermented Vegetables as Potential Probiotics. *Kasetsart J. (Nat. Sci.)*, 2011, 45, 1071-1082. Available online: <https://www.thaiscience.info/journals/Article/TKJN/10898384.pdf> (accessed on 10 July 2024).
- [16] Concise Review: Importance of Probiotics Yogurt for Human Health Improvement. Available online: https://www.researchgate.net/publication/280566268_Concise_Review_Importance_of_Probiotics_Yogurt_for_Human_Health_Improvement (accessed on 10 July 2024).
- [17] Goldenberg, J.Z.; Yap, C.; Lytvyn, L.; Lo, C.K.; Beardsley, J.; Mertz, D.; Johnston, B.C. Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children. *Cochrane Library*, 2017, 12, DOI: <https://doi.org/10.1002/14651858.cd006095.pub4>.
- [18] Plessas, S., Bosnea, L., Alexopoulos, A., & Bezirtzoglou, E. (2012). Potential effects of probiotics in cheese and yogurt production: A review. *Engineering in Life Sciences*, 12(4), 433-440. DOI: <https://doi.org/10.1002/elsc.201100122>.
- [19] Carroll, K.C.; Mizusawa, M. Laboratory Tests for the Diagnosis of Clostridium difficile. *Clinics in Colon and Rectal Surgery*, 2020, 33(02), 073-081. DOI: <https://doi.org/10.1055/s-0039-3400476>.
- [20] Szajewska, H.; Canani, R.B.; Guarino, A.; Hojsak, I.; Indrio, F.; Kolacek, S.; Orel, R.; Shamir, R.; Vandenplas, Y.; Van Goudoever, J.B.; Weizman, Z. Probiotics for the prevention of Antibiotic-Associated diarrhea in children. *Journal of Pediatric Gastroenterology and Nutrition*, 2015, 62(3), 495-506. DOI: <https://doi.org/10.1097/mpg.0000000000001081>.
- [21] Compound Eosinophil-Lactobacillus Tablets Instruction-caution-adverse effect-dosage and administration-39 Yaopintong. (n.d.). Available online: <https://ypk.39.net/714808/manual/> (accessed on 14 July 2024).

- [22] Szajewska, H.; Canani, R.B.; Guarino, A.; Hojsak, I.; Indrio, F.; Kolacek, S.; Orel, R.; Shamir, R.; Vandenplas, Y.; Van Goudoever, J.B.; Weizman, Z. Probiotics for the prevention of Antibiotic-Associated diarrhea in children. *Journal of Pediatric Gastroenterology and Nutrition*, 2015, 62(3), 495-506. DOI: <https://doi.org/10.1097/mpg.0000000000001081>.
- [23] Bacillus Licheniformis Capsule, Live_effect_instruction|dxy. (2018). Available online: <https://dxy.com/drug/10348/detail> (accessed on 15 July 2024).
- [24] Baral, K.C.; Bajracharya, R.; Lee, S.H.; Han, H. Advancements in the pharmaceutical applications of probiotics: dosage forms and formulation technology. *International Journal of Nanomedicine*, 2021, 16, 7535-7556. DOI: <https://doi.org/10.2147/ijn.s337427>.



© 2024 by the author(s); licensee International Technology and Science Publications (ITS), this work for open access publication is under the Creative Commons Attribution International License (CC BY 4.0). (<http://creativecommons.org/licenses/by/4.0/>)