

Is There a Need for Regular Surveillance for Bacterial Contaminants in Street Foods of Kathmandu?

Bhuvan Saud ^{1*}, Pradeep Devkota ¹, Govinda Paudel ¹, Neetu Amatya ¹, Vikram Shrestha ¹, Ashish Lakhey ², Radha Ranabhat ¹

¹ Department of Medical Laboratory Technology, Janamaitri Foundation Institute of Health Sciences, Hattiban, Lalitpur, Nepal.

² Department of Pathology, Kist Medical College and Hospital, Lalitpur, Nepal.

*Corresponding Author: Bhuvan Saud, Department of Medical Laboratory Technology, Janamaitri Foundation Institute of Health Sciences, Hattiban, Lalitpur, Nepal.

Received date: March 23, 2023; Accepted date: April 06, 2022; Published date: April 17, 2023

Citation: Saud B., Devkota P., Paudel G., Amatya N., Shrestha V., (2023), Is There a Need for Regular Surveillance for Bacterial Contaminants in Street Foods of Kathmandu? *Clinical Research and Studies*, 2(1) DOI:[10.31579/2835-2882/017](https://doi.org/10.31579/2835-2882/017)

Copyright: © 2023, Bhuvan Saud. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: Ready-to-eat street food is common around the world. Unsafe food becomes a source of infection for consumers. This study aimed to investigate the status of bacterial contaminants, antibiotic-resistant patterns and hygiene practices among street-food vendors in Kathmandu, Nepal.

Methodology: A total of 50 ready-to-eat food samples (Momo with soup, Smashed potato, Panipuri, Watermelon and Papaya) were collected from inside Kathmandu city. Collected samples were then cultured in different agar mediums and incubated under controlled laboratory conditions. Bacterial identification was performed via colony morphology, staining and biochemical tests. Identified bacteria were subjected for antibiotic susceptibility test and hygiene practices data were also collected.

Results: Out of 50 samples, the prevalence of bacterial isolates was 70 with six distinct bacterial species identified. The mean bacterial count in ready-to-eat momo with soup was 4.8 X10⁶ cfu/g and mean 4.2 X 10⁶ cfu/g in crushed potato. Polymicrobial growth was observed in more than 62.0% of samples. Momo and Papaya contained the equally highest prevalent of bacterial isolates and Panipuri the least contaminated. Overall, the predominant organism was *Staphylococcus aureus* followed by *Klebsiella pneumoniae*, *Citrobacter* species, and *Escherichia coli*. Most of the isolates were resistant to Amoxicillin whilst 3 isolates were multidrug resistant. On the other hand, *Salmonella* species and *Acinetobacter* species were sensitive to all the tested antibiotics. Also, there was a lack of hygiene practices with a dearth of basic equipment and infrastructure.

Conclusion: This indicates that prompt and periodic quality monitoring and bacteriological examination of ready-to-eat street foods of Kathmandu is utmost necessary.

Keywords: street food; momo; fruits, *staphylococcus aureus*; *klebsiella pneumoniae*; antibiotic resistance

Introduction

According to the Food and Agriculture Organization (FAO) street food is “ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers especially in streets and other similar public places”[1]. Street-food eating practice is prevalent in every corner of the world. However, consumption of unsafe food leads to acute to long term diseases, disabilities and deaths. Globally, one in ten people becomes ill due to the consumption of contaminated food [2]. Microorganisms such as non-typhoidal *Salmonella enterica*, *Salmonella Typhi*, *Taenia solium*, Hepatitis A virus, enteropathogenic *Escherichia coli* and aflatoxin are mainly responsible for foodborne illness [3]. Data shows that 41 and 200 people were severely affected due to consumption of contaminated food and water respectively in Nepal [4,5] and the burden associated with

consumption of unsafe food has been reported elsewhere at different time periods [6-8].

Nowadays, street-food has become the first choice for the customers due to its low cost, easy availability and taste. In developing countries, street foods are consumed by millions of people [6,9]. Likewise, inside Kathmandu valley, over 30 thousand street-food stalls serve food everyday [10]. Poor preparation practice, improper handling, lack of personal hygiene, insufficient awareness, lack of cold storage facilities, inadequate clean water supply for cleaning and drinking purposes are the prominent culprits for transmission of pathogenic microorganism. Also, drug-resistant superbugs have been identified in street meals [11]. Multitude research articles from different countries like Ethiopia [12,13], Bangladesh [14,15], India [16], and Nepal [17] advocate that the food we

consume acts as a vehicle for pathogen transmission. Meanwhile, contaminated water supply [18] and raw food [19] are the vital source of pathogens even if all preventive measures are taken by vendors. Annually, around 0.4 million people succumb due to consumption of unsafe food [2]. These circumstances pose an additional serious threat for general public. In Kathmandu, millions of people daily consume ready-to-eat street foods. However, its quality of is unknown and very limited reports have been published regarding the same. Hence, the present study is designed to investigate the bacterial prevalence, antibiotic resistance pattern and hygiene practices in street foods inside Kathmandu.

Methodology

A cross-sectional study was conducted from August 2019 to January 2020 (6 months). A total of 50 ready to eat food samples (Momo with soup, Panipuri, Smashed potato, Watermelon and Papaya) were collected from street vendors at different locations of ring road (around 27 kilometers range) in Kathmandu via convenient sampling technique. Informed verbal consent was taken from street-vendors. About 10 gram of each sample was collected in a sterile leak-proof container, and transported maintaining cold chain to Department of Medical Laboratory Technology, Janamaitri Foundation Institute of Health Science, Hattiban. Samples were then homogenized for 2 minutes in 9 ml of 1% phosphate buffer saline and vortex to dislodge adhered bacteria. Exactly 0.1ml of sample was transferred into Blood agar, Mac-conkey agar, and Mannitol

salt agar and incubated in aerobic condition at 37°C for 24 hours. Isolated bacteria were further identified by their colony, staining and biochemical characteristics. The isolated bacteria were subjected for antimicrobial susceptibility testing in Mueller hinton agar as per the Clinical and Laboratory Standards Institute guidelines [20]. Furthermore, antimicrobial agents were selected based on the availability and frequency of prescription for the treatment of bacterial infections by clinicians in Nepal. ATCC 25922 *Escherichia coli* was used as reference organism for quality control. The obtained data were entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 20 and interpreted according to frequency distribution and percentage table.

Results

Out of 50 samples, 70 bacterial isolates were detected with six distinct bacterial species. *Staphylococcus aureus* was the most commonly isolated organism followed by *Klebsiella pneumoniae*, *Citrobacter* species and *Escherichia coli*. *Proteus* species and *Salmonella* species were the least isolates seen so far. Ready-to-eat Momo with soup accounted for the highest mean bacterial count (4.8 X10⁶cfu/gm) followed by Watermelon (mean 4.0 X10⁵cfu/gm), Panipuri (mean 3.2 X10⁵cfu/gm), and Papaya (mean 2.8 X10⁵cfu/gm) as shown in Table 1.

Organisms	Momo	Panipuri	Potato	Watermelon	Papaya	Total isolates
<i>E. coli</i>	3	1	2	2	1	9
<i>K. oxytoca</i>	3	0	0	1	3	7
<i>K. pneumoniae</i>	2	3	3	4	4	16
<i>Citrobacter</i> species	3	2	1	2	3	11
<i>Salmonella</i> species	0	0	1	0	0	1
<i>Acinetobacter</i> species	0	0	0	1	1	2
<i>Proteus</i> species	1	0	0	0	0	1
<i>Staphylococcus aureus</i>	5	3	5	5	5	23
Total isolates	17	9	12	15	17	70
Mean bacterial count (CFU/g)	4.8 X10 ⁶	3.2 X10 ⁵	4.2 X 10 ⁶	4.0 X10 ⁵	2.8 X10 ⁵	-

Table 1: Distribution of isolates from the samples

Overall, in this study, Amoxicillin resistant organisms were found to be most prevalent one. *Salmonella* species and *Acinetobacter* species were sensitive to all the antibiotics. Surprisingly, all the isolated *Klebsiella*

oxytoca were resistant to Amoxicillin, and 50.0% isolates were resistant to Nitrofurantoin. In *Staphylococcus aureus*, out of 23 isolates, 22 were resistant to Amoxicillin of which two were MDR. Also, one isolate of *E. coli* was MDR (Table 2).

Organisms	AMX	CFX	GM	TE	NIT	COT	CHL	CEX	E	CM
<i>E. coli</i> (9)	8 (88.9%)	0 (0.0%)	1 (11.1%)	3 (33.3%)	1 (11.1%)	0	0	-	-	-
<i>K. pneumoniae</i> (7)	6 (85.7%)	1 (14.2%)	0 (0.0%)	0	2 (28.6%)	0	0	-	-	-
<i>K. oxytoca</i> (16)	16 (100.0%)	3 (18.7%)	2 (12.5%)	0	8 (50.0%)	1 (6.2%)	3 (18.8%)	-	-	-
<i>Citrobacter</i> species (11)	10 (90.9%)	0	0	0	4 (36.4%)	0	0	-	-	-
<i>Salmonella</i> species (1)	0	0	0	0	0	0	0	-	-	-
<i>Acinetobacter</i> species (2)	0	0	0	-	0	-	-	-	-	-
<i>Proteus</i> species (1)	0	0	0	0	0	0	0	-	-	-
<i>Staphylococcus aureus</i> (23)	22 (95.7%)	-	0	2 (8.7%)	-	2 (8.7%)	-	4 (17.4%)	8 (34.8%)	0

Note: *Acinetobacter* species were tested for Ampicillin sulbactam, Imipenem and Ciprofloxacin; AMX, Amoxicillin; E, Erythromycin; COT, Cotrimoxazole; TE, Tetracycline; NIT: Nitrofurantoin; CEX, Cefoxitin; CM, Clindamycin; CFX, Ceftazidime; CHL, Chloramphenicol; GM, Gentamycin; NA, Nalidixic acid; NOR, Norfloxacin; - = not tested

Table 2: Antibiotic-resistant pattern of isolated organism

In this study, 62.0% of vendor personnels did not maintain nail hygiene and 96.0% worked with bare hands and 100.0% without food safety training. Majority of the vendors mobile, i.e. not fixed in a particular

location. Also, there was a lack of proper freezer or cold storage, water supply, closed dustbins, hand washing facility, utensils cleaning, and covered facilities (Table 3).

Practices	Status	Frequency (n)	Percentage (%)
Nail hygiene	Yes	19	38.0%
	No	31	62.0%
Glove use	Yes	2	4.0%
	No	48	96.0%
Cold storage facility	Yes	1	2.0%
	No	49	98.0%
Water supply	Yes	6	12.0%
	No	44	88.0%
Waste disposal facility	Yes	28	56.0%
	No	22	44.0%
Workers cloth hygiene	Yes	33	66.0%
	No	17	44.0%
Hand washing facility for consumers	Yes	7	14.0%
	No	43	86.0%
Utensils clean and covered	Yes	13	26.0%
	No	37	74.0%
Pest and flies control	Yes	12	24.0%
	No	38	76.0%
Food safety training	Yes	0	0.0%
	No	50	100.0%
Literacy	Illiterate	21	42.0%
	Literate	29	58.0%
Food stall type	Fixed	8	16.0%
	Mobile	42	84.0%

Table 3: Food handling and hygiene practices by vendors

Discussion

Several factors such as microorganisms, toxins, heavy metals, chemicals, pesticides, etc. make food unsafe to consume [21]. The trend of consumption of street food is escalating tremendously all over the world due to its easy accessibility, inexpensive and attractive nature [22]. Globally, food-associated illness accounts for significant morbidity and mortality. The burden of food-borne diseases is reported higher in developing countries [3]. However, all the microorganisms isolated from street-food may not cause serious illness. Out of 70 bacterial isolates, 32 isolates were detected from Fruit salad with 15 from Watermelon and 17 from Papaya. *S. aureus* and *K. pneumonia* was most commonly found bacteria from the fruits. In this study, foodborne pathogens like *E. coli*, *S. aureus*, *Citrobacter* spp., *Salmonella* spp., *Klebsiella* spp., and *Proteus* spp. were isolated. Among them *S. aureus* was the most predominant bacteria (32.8%) and 6.2% isolates were MDR. A study from India have reported virulent methicillin-resistant *S. aureus* from street-vended foods [23]. Similarly, a study conducted in Chitwan, Nepal revealed that out of 50 *S. aureus* isolates, 4 (8.0%) isolates were MDR, and out of 30 (36.6%) *E. coli* isolates, 11 were MDR bacteria. Overall, out of 164 bacteria isolates, 29 (14.6%) were MDR isolates [17]. Likewise, a study conducted in Rishikesh, India, in street fruits, the most common bacteria were *Klebsiella* spp., *E. coli*, *Citrobacter* spp., *P. aeruginosa*, *S. aureus*, *Streptococcus* spp., and *Enterococcus* spp., but they had not isolated a single *Salmonella* spp. from fruits which is similar to our result [24]. However, we found one isolates of *Salmonella* spp. from smashed potato. Different studies conducted around the world advocate that the variety of pathogens are predominant in street foods [25-30].

Food handling, hygiene status and appliances were seemed to be compromised by the street vendors. The data showed that vendors did not maintain nail hygiene (62.0%), working with bare, hands (96.0%), lack of cold storage (98.0%), lack of water supply (88.0%), lack of clean cloth hygiene (66.0%) and lack of food safety training (100.0%) that may directly affect the quality of food (Table 3). A study from Zimbabwe revealed that the food is mainly contaminated during the handling or processing [31]. Also, a study from Colombia unveiled that 30.0% of food handlers carry pathogenic bacteria such as *S. Typhi*, *S. aureus*, *S. enteritidis*, and *Shigella* [32]. Likewise, lack of a proper cold storage facility could escalate the microbial population in foods. Other findings showed that bacterial count increased 1-3 log cycles after 16 and 24 hours

in ambient temperature [33]. Food handlers also transmit microorganisms from faces, nose and skin into the food [34]. It has been found that *Salmonella* spp., non-typhi salmonella, *Campylobacter* and *E. coli* could even survive on the fingertips [35].

On the other hand, the location of food stall may also affect the quality of food. In Kathmandu, most of the street-food stalls are mobile and some are established nearby garbage or dumping sites. Environmental conditions also play a prominent role in food quality. Uncovered foods, uncontrolled flies, and wind-dust could deteriorate the food. In Africa, 75.0% vendors prepare foods in unhygienic conditions [36]. Meanwhile, semi-cooked or uncooked eating tradition in some food items also increases the probability of infection. Additionally, supply of contaminated drinking water with *E. coli*, Aichi virus-1, Human Adenoviruses, Enteroviruses and Noroviruses in Kathmandu become a offender for pathogen transfer [37]. In Kathmandu valley, around 92.0% of jar water, 77.0% of tanker-water and 69.0% of filtered water are unsuitable for drinking purpose due to soaring number of coliforms [18]. Therefore, lack of knowledge, practice, sanitation, utensils and infrastructure makes the street foods more vulnerable.

In this light, Government and local personnels should take responsibility for its legislation and implementation of effective policy in this sector by providing adequate facilities, periodic monitoring of food quality, sanitation training to the street-food vendor and minimize the irrational use of antibiotics in food products in order to reduce the burden of food-borne diseases and to improve the health of consumers [38-41].

Conclusion

In this study, six distinct types of bacterial species were isolated and few of them were multidrug resistant. Higher number of bacteria in ready-to-eat food may persuade a serious life-threatening burden to the consumers. Thus, there is a need of decisive action and periodic surveillance to improve the quality of street foods.

Abbreviations

Spp: Species

MDR: Multidrug resistant

ATCC: American Type Culture Collection

Funding:

No funding received from governmental or public sectors.

Conflict of interest:

We do not have any conflict of interest.

Authors' contribution

B S designed the study. B S, P D, G P, NA carried out data collection, laboratory work, and data analysis. B S, G P, NA prepared the manuscript. VS, AL and R B made critical comment on the manuscript. All the authors finalized and approved the manuscript.

References

- Fellows P, Hilmi M. (2011). Selling Street and snack foods. *FAO diversification booklet*. (18).
- World Health Organization (WHO). Food safety.
- World Health Organization. (2015). WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. *World Health Organization*.
- Himalayan News Service. (2018). 200 taken ill due to food poisoning. *The Himalayan Times*.
- Dhakal S. (2012). 22 admitted to hospital after food poisoning. *The Himalayan Times*.
- Food and Agriculture Organization of the United Nations. (1990). Street Foods-Report of an FAO Expert Consultation Yogyakarta, Indonesia, 5-9.
- Estrada-Garcia T, Lopez-Saucedo C, Zamarripa-Ayala B, Thompson MR, Gutierrez-Cogco L, et al. (2004). Prevalence of *Escherichia coli* and *Salmonella* spp. in street-vended food of open markets (tianguis) and general hygienic and trading practices in Mexico City. *Epidemiology & Infection*. 132(6):1181-1184.
- Ghosh M, Wahi S, Kumar M, Ganguli A. (2007). Prevalence of enterotoxigenic *Staphylococcus aureus* and *Shigella* spp. in some raw street vended Indian foods. *International Journal of Environmental Health Research*. 17(2):151-156.
- Sanlier N, Sezgin AC, Sahin G, Yassibas E. (2018). A study about the young consumers' consumption behaviors of street foods. *Ciencia & saude coletiva*. 23:1647-1656.
- Adhikari DB. (2011). Income generation in informal sector: A case study of the street vendors of Kathmandu Metropolitan City. *Economic Journal of Development Issues*. 1-4.
- Amare A, Worku T, Ashagirie B, Adugna M, Getaneh A, et al. (2019). Bacteriological profile, antimicrobial susceptibility patterns of the isolates among street vended foods and hygienic practice of vendors in Gondar town, Northwest Ethiopia: a cross sectional study. *BMC microbiology*. 19(1):1-9.
- Eromo, T., Tassew, H., Daka, D., & Kibru, G. (2016). Bacteriological quality of street foods and antimicrobial resistance of isolates in Hawassa, Ethiopia. *Ethiopian journal of health sciences*, 26(6):533-542.
- Tadesse G, Mitiku H, Teklemariam Z, Marami D. (2019). *Salmonella* and *Shigella* among asymptomatic street food vendors in the Dire Dawa city, Eastern Ethiopia: prevalence, antimicrobial susceptibility pattern, and associated factors. *Environmental health insights*. 13:1178630219853581.
- Tabassum A, Saha ML, Islam MN. (2015). Prevalence of multi-drug resistant bacteria in selected street food and water samples. *Bangladesh Journal of Botany*. 44(4):621-627.
- Dey M, Mokbul M, Ismail I, Alim SR. (2018). Identification of Antibiotic Resistant Gram-Negative Bacteria in a Popular Street-Food Item (Chatpati) in Dhaka University Campus, Bangladesh. *FEM*. 4:75-80.
- Shagufta B, Sivakumar M, Kumar S, Agarwal RK, Bhilegaonkar KN, et al. (2017). Antimicrobial resistance and typing of *Salmonella* isolated from street vended foods and associated environment. *Journal of food science and technology*. 54(8):2532-2539.
- Khadka S, Adhikari S, Rai T, Ghimire U, Parajuli A. (2018). Bacterial contamination and risk factors associated with street-vended Panipuri sold in Bharatpur, Nepal. *Int J Food Res*. 5:32-38.
- Maharjan, S., Joshi, T. P., & Shrestha, S. M. (2018). Poor quality of treated water in Kathmandu: Comparison with Nepal drinking water quality standards. *Tribhuvan University Journal of Microbiology*. 5: 83-88.
- Saud, B., Paudel, G., Khichaju, S., Bajracharya, D., Dhungana, G., et.al (2019). Multidrug-resistant bacteria from raw meat of buffalo and chicken, Nepal. *Veterinary medicine international*.
- Clinical and Laboratory Standards Institute (CLSI). (2017). Performance Standards for Antimicrobial Susceptibility Testing 27th ed. CLSI supplement 100.
- Imathlu, S. (2017). Street Vended Foods: Potential for Improving Food and Nutrition Security or a Risk Factor for Foodborne Diseases in Developing Countries? *Current Research in Nutrition and Food Science Journal*, 5(2): 55-65.
- Draper A. (1996). Street foods in developing countries: the potential for micronutrient fortification. John Snow, Incorporated, OMNI PROJECT.
- Sivakumar, M., Dubal, Z. B., Kumar, A., Bhilegaonkar, K., Kumar, O. R. V., et.al., (2019). Virulent methicillin resistant *Staphylococcus aureus* (MRSA) in street vended foods. *Journal of food science and technology*, 56(3):1116-1126.
- Malik, Y., Omar, B. J., & Singh, A. (2020). Bacteriological analysis of street-vended fruit juices available in Rishikesh, Uttarakhand. *Journal of Family Medicine and Primary Care*, 9(2):938.
- Ma L, Chen H, Yan H, Wu L, Zhang W. (2019). Food safety knowledge, attitudes, and behavior of street food vendors and consumers in Handan, a third-tier city in China. *BMC public health*. (1):1-3.
- Bryan, F. L., Jermini, M., Schmitt, R., Chilufya, E. N., Michael, M., et.al. (1997). Hazards associated with holding and reheating foods at vending sites in a small town in Zambia. *Journal of Food Protection*, 60(4):391-398.
- Bryan FL, Teufel P, Riaz S, Roohi S, Qadar F, et al. (1992). Hazards and critical control points of street-vended chat, a regionally popular food in Pakistan. *J Food Prot*. 55:708-713
- Mosupye FM, Holy A. (1999). Microbiological quality and safety of ready to-eat street-vended foods in Johannesburg, South Africa. *J Food Prot*. 62:1278-1284.
- Muleta D, Ashnafi M. (2001). *Salmonella*, *Shigella* and growth potential of other food-borne pathogens in Ethiopian street vended foods. *East Afr Med J*. 78(11):576-580.
- Umoh VJ, Odoba MB. (1999). Safety and quality evaluation of street foods sold in Zaire, Nigeria. *Food Control*. 10:9-14
- Gumbo, A., Bangure, D., Gombe, N. T., Tshimanga, M., Hwalima, Z., (2015). *Staphylococcus aureus* food

- poisoning among Bulawayo City Council employees, Zimbabwe, 2014. *BMC research notes*, 8(1): 1-6.
32. Rane, S. (2011). Street vended food in developing world: hazard analyses. *Indian journal of microbiology*, 51(1):100-106.
 33. Kaul M, Agarwal G. (1989). Microbial load of common chat products. *Indian J Nutr Diet*.1988;25:197-199
 34. World Health Organization (WHO). Health surveillance and management procedures for food handling personnel. *WHO technical report series*. 785.
 35. Pether JV, Gilbert RJ. (1971). The survival of salmonellas on finger-tips and transfer of the organisms to foods. *Epidemiology & Infection*. 69(4):673-681.
 36. Muinde OK, Kuria E. (2005). Hygienic and sanitary practices of vendors of street foods in Nairobi, Kenya. *Afr J Food Agric Nutr Dev*. 5(1):1-14.
 37. Malla, B., Ghaju Shrestha, R., Tandukar, S., Bhandari, D., Thakali, O., et.al. (2019). Detection of pathogenic viruses, pathogen indicators, and fecal-source markers within tanker water and their sources in the Kathmandu Valley, Nepal. *Pathogens*, 8(2), 81.
 38. Tuladhar, R., & Singh, A. (2012). Bacterial Analysis and Survey of the Street Food of Kathmandu in Relation to Child Health. *Journal of Natural History Museum*, 26:1-9.
 39. Bhaskar, J., Usman, M., Smitha, S., & Bhat, G. K. (2004). Bacteriological profile of street foods in Mangalore. *Indian journal of medical microbiology*, 22(3): 197.
 40. Adhikari, S., Saud, B., Paudel, G., & Bajracharya, D. (2019). Emergence of Antimicrobial Drug Resistant Bacteria in Nepal: A Current Scenario. *Proteomics Bioinformatics Current Res*, 1(1), 31-33.
 41. Saud, B., Pandey, P., Paudel, G., Dhungana, G., & Shrestha, V. (2020). In-vitro Antibacterial Activity of Probiotic against Human Multidrug Resistant Pathogens. *Archives of Veterinary Science and Medicine*. 3: 31-39.

Ready to submit your research? Choose ClinicSearch and benefit from:

- fast, convenient online submission
- rigorous peer review by experienced research in your field
- rapid publication on acceptance
- authors retain copyrights
- unique DOI for all articles
- immediate, unrestricted online access

At ClinicSearch, research is always in progress.

Learn more <https://clinicsearchonline.org/journals/clinical-research-and-studies->



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.