Prithvi Journal of Research and Innovation
[A Peer-Reviewed, Open Access Multidisciplinary Bilingual Journal; Indexed in NepJOL]
ISSN 2705-4888 [Print]; ISSN 2705-4896 [Online]; JPPS Star-Rated Journal
Volume 7; 15 December 2025; pp. 1-14
eJournal Site: http://ejournals.pncampus.edu.np/ejournals/pjri/

Research Article

Bacteriological Assessment of Ready-to-Eat Noodles and Accompanying Seasonings in Nepal: Implications for Food Safety and Public Health

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Article History:
Submitted 08 October 2025
Reviewed 31 October 2025
Revised 05 November 2025
Accepted 01 December 2025

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Article DOI:
https://doi.org/10.3126/pjri.v7i1.87669

Publisher:
Centre for Research and Innovation
Prithvi Narayan Campus
Tribhuvan University, Pokhara, Nepal
[Accredited by UGC, Nepal]

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ABSTRACT

The purpose of this study was to evaluate the bacteriological quality of ready-to-eat (RTE) instant noodles and their accompanying seasonings vended in local marketplaces of Pokhara, Nepal. A total of 66 samples, comprising 33 instant noodle packages and thirty-three seasoning sachets from eleven different brands (A, B, C, D, E, F, G, H, I, J and K) were analysed for bacterial contamination using Plate Count agar to enumerate total viable bacteria. Among the noodle samples, brand A exhibited the highest contamination level with a bacterial count of 3.03×10^5 CFU/gram, followed by brand C with bacterial count of 2.99×10^5 CFU/gram. For the seasonings, brand K had the highest level of contamination with a bacterial count of 1.65×10^6 CFU/gram, followed by the seasoning from brand A with count of 8.97×10^5 CFU/gram. Of the total 59 bacterial isolates obtained, 94.92% were gram-positive, including *Staphylococcus aureus* (44.1%), *Bacillus* spp (32.2%), and Coagulase negative *Staphylococci* (*CoNS*) (18.6%) and gram-negative bacteria represented by only *Klebsiella* spp (5.1%). Antibiotic susceptibility tests (AST) showed that all the isolates of *CoNS*, *Klebsiella* species, and *Staphylococcus aureus* were 100% susceptible to Co-Trimoxazole, Gentamicin, Doxycycline, and Chloramphenicol. All isolates obtained exhibited complete resistance to Ampicillin, whereas *Klebsiella* spp isolates also showed resistance to Azithromycin. These findings revealed a significant microbial load and antibiotic-resistant bacteria in RTE instant noodles, highlighting the need for improved hygiene practices and regular monitoring of microbial and antibiotic resistance patterns in ready-to-eat instant noodles, particularly

in the seasoning sachets, to safeguard public health.

KEYWORDS: Ready-to-eat noodles, seasonings, bacterial contamination, antibiotic resistance

INTRODUCTION

The consumption of ready-to-eat (RTE) foods, and instant noodles in particular, has rapidly increased in developing countries such as Nepal due to factors like better online accessibility, lower prices, longer shelf life, and convenience of cooking in urban and semi-urban areas (Kim et al., 2017; Ghimire et al., 2019). Their main ingredients are wheat flour and palm oil fat, but they also contain other components and additives, and they may be sold cooked (steamed) and then deep-fried, or dried without cooking. They are often sold in packets containing flavouring agents, such as powdered soup mixes, dried vegetables, and oils that enhance flavour and aroma (Lee, 2009; FAO, 2018), contributing their widespread popularity as a convenient meal option.

Globally, millions of packs of instant noodles are consumed every year, making it the largest market. Nepal ranked third in per capita consumption in 2021, at 55 servings per person per year, after Vietnam and South Korea (WINA, 2024). If improperly handled and processed, noodles could be contaminated with pathogenic bacteria such as *Salmonella* spp, *Shigella* spp, *Staphylococcus aureus*, and *Escherichia coli*, thereby posing health risks to susceptible individuals (Mohinudeen et al., 2024; Bhoomika & Kumar, 2023). Many noodle snacks sold in markets in Kathmandu were found to contain pathogenic microorganisms (Arjyal et al., 2023). Similar problems were observed in ready-to-eat instant noodles in other countries, such as Indonesia and Sri Lanka, due to inadequate hygiene and a lack of regulatory oversight (Siti et al., 2020; Mohinudeen et al., 2024) and emphasize the global importance of maintaining strict food safety standards.

Young people and working adults often choose instant noodles for their low cost and convenience, and they are usually consumed by simply boiling or reheating (Lee et al., 2018). However, they consistently provide an imbalanced nutritional profile: high in carbohydrates, saturated fat, and sodium, and low in fiber, vitamins, and minerals. Increased risk of conditions such as metabolic syndrome, high blood pressure, and digestive problems was associated with the consumption of instant noodles (Wina et al., 2020; Shin et al., 2014). Despite the increased popularity of ready-to-eat instant noodles, there has been a slight improvement in their safety, raising questions about the sanitary conditions of those commonly sold in markets without proper labelling or hygienic packaging (WHO, 2022) which underscores the need for stronger quality control measures.

In addition to the nutritional components of instant noodles, their microbial safety has become a significant concern. This includes the safety of the flavouring packet, given the risk of contamination during manufacturing, packaging, and storage. Instant noodles should not support the growth of any microbes due to their lower water activity. However, seasonings, particularly those with an oil base or of animal origin, may support the growth of specific microorganisms (Nguyen et al., 2021). In addition, those are added after cooking, which is not a sufficient heat treatment to eliminate microbial contamination.

Inadequate hygiene during production, packaging, or handling could contribute to the presence of potentially pathogenic bacteria in ready-to-eat foods, some of which may also exhibit antibiotic resistance (Budathoki et al., 2021). Antibiotic-resistant foodborne bacteria posed the threat of causing disease and compromising treatment. Reports from Bangladesh and India have identified enteric pathogens with

multidrug resistance in ready-to-eat street foods, reflecting unregulated antibiotic use (Srivastava et al., 2022). RTE noodles and their seasoning mixes are consumed in great quantities, and some studies have assessed the microbial safety of ready-to-eat foods, but there is a lack of research focusing particularly on the prevalence of antibiotic-resistant bacteria in commercially packaged instant noodles and their seasoning sachets, particularly in Pokhara, Nepal. To fill this neglected yet critical research gap, this study hypothesized that commercially available RTE instant noodles and their accompanying seasonings sold in Pokhara would harbor antibiotic-resistant bacteria. It aimed to evaluate not only the bacteriological quality but also the antibiotic resistance profiles of bacterial isolates obtained from ready-to-eat instant noodles and seasonings available in the local markets of Pokhara, Nepal. The findings of the research will be informative for authorities in strengthening food safety monitoring and surveillance of antibiotic resistance.

RESEARCH METHODS

A cross sectional descriptive study was conducted involving eleven different brands of ready-to-eat instant noodles commonly available in Pokhara, Nepal, which were collected from two large supermarkets in Pokhara, Nepal: Big Mart (Bagar) and Bhatbhateni Supermarket (New Road). The selected brands were coded as A, B, C, D, E, F, G, H, I, J, and K. For each brand, three individual packets were collected and used as samples. From each packet, the raw noodle portion and its accompanying seasoning sachet were separated and analysed as independent samples. This resulted in 33 raw noodle samples and 33 seasoning packet samples, making up a total sample size of 66. Collected samples were transferred to the Microbiology laboratory of Prithvi Narayan Campus and samples were processed there. The sachets were thoroughly inspected to ensure they were intact and within their expiration date before being taken for analysis.

Samples Processing and Preparations

For homogenization, 1 gram of each noodle along with their corresponding seasonings, was weighed separately and well mixed with 9 ml of normal saline using a sterile mortar and pestle. All the samples were mixed thoroughly before further examination.

Isolation and Enumeration of Bacteria in Noodles and Seasonings Samples

The Spread Plate Technique was used to determine the microbial load in each sample. 1 ml of each sample was placed into a test tube containing 9 ml of sterile saline at a 9:1 ratio. Then serial dilutions were prepared to the 10^{-4} dilution according to the standard method. 0.1 ml of the 10^{-1} and 10^{-3} dilutions was then inoculated into Plate Count Agar using the spread plate technique and incubated at 37 °C for 24 hours (Chesbrough, 2006). The average counts for triplicate cultures were recorded as the bacterial counts in the sample. The total bacterial counts from the samples were expressed as the number of organisms or Colony Forming Units per gram (CFU/gram). The colony count in CFU/g was obtained using the formula:

CFU/g = (Number of Colonies Counted x Dilution factor)/ Volume Plated

Isolation and Presumptive Identification of Bacteria in Noodles and Seasonings Samples

Each sample from the original homogenized suspension was plated onto Mannitol Salt agar, MacConkey agar, and Nutrient agar, and incubated at 37 °C for 24-48 hours. After incubation, the resulting colonies were observed, subjected to Gram staining, and subsequently subcultured onto Nutrient Agar to obtain pure cultures following 37°C for 24 hours (Chesbrough, 2006). After incubation, the subcultured pure-culture colonies were again subjected to Gram staining to determine suitable

biochemical test methods. For Gram-positive cocci, Catalase and Coagulase tests were conducted. For Gram-negative bacilli, Sulphide-Indole-Motility (SIM) test, Methyl Red-Voges Proskauer (MR-VP) test, Citrate utilization test, Triple Sugar Iron (TSI) test, Urease test, etc, were carried out following standard microbiological techniques as described by Chesbrough (2006).

Antibiotic Susceptibility Tests

An antibiotic susceptibility test was performed using the *Staphylococcus aureus*, Coagulase-negative Staphylococci (*CoNS*), and the *Klebsiella* spp strains isolated from the different samples. Bacterial suspensions were prepared, and the turbidity was set to match a 0.5 McFarland standard. The suspensions were then evenly spread across the surface of Muller-Hinton agar (Hi-media), and Chloramphenicol (30 µg), Doxycycline Hydrochloride (30 µg), Gentamycin (10 µg), Co-Trimoxazole (25 µg), Azithromycin (15 µg), and Ampicillin (10 µg) were placed on the surface of the agar. Then, the plates were incubated at 37 °C for 18 hours, and the diameters of the inhibition zones were measured (CLSI, 2023; EUCAST, 2023).

Data Collection and Analysis

Descriptive statistics were used to analyse the distribution, frequency, and prevalence of bacterial species in noodle and seasoning samples. Microbial load was expressed as colony-forming units per gram (CFU/g), with means shown. Analysis of Variance (ANOVA) was performed to determine whether there was a significant difference in contamination levels among noodles and seasonings; the significance level was set at $p<0.05$.

RESULTS

A total of 66 samples, comprising 33 instant noodle packages and thirty-three seasoning sachets from eleven different brands (A, B, C, D, E, F, G, H, I, J and K) were analysed for bacterial contamination using Plate Count agar to enumerate total viable bacteria. The total bacterial counts from the samples were expressed as the number of organisms or Colony Forming Units per gram (CFU/gram).

Total Viable Bacterial Load in Instant Noodle Samples

Among the noodle samples, brand A had the highest bacterial load, averaging 3.03×10^5 CFU/g, followed closely by brand C at 2.99×10^5 CFU/g, indicating higher bacterial levels in both brands. Brands D and E had an average count of 1.00×10^4 CFU/g. Brand B showed lower contamination, with an average of 1.70×10^5 CFU/g. Likely, brands I and K demonstrated moderate contamination levels, with mean counts of 2.97×10^4 CFU/g and 3.86×10^4 CFU/g, respectively. Brand F and H exhibited very low levels of contamination, with mean values of 2.50×10^1 CFU/g and 1.0×10^1 CFU/g, respectively. Notably, brand G had the lowest bacterial counts, averaging 0.50×10^1 CFU/g (Table 1).

Seasoning samples exhibited higher levels of microbial count than those of noodle samples. Brand K exhibited the highest bacterial load, with an average CFU/g of 1.65×10^6, followed by brands A and C, with average values of 8.97×10^5 CFU/g and 5.71×10^5 CFU/g, which were possibly not safe levels of microbial contamination in the seasoning samples. Moderate contamination was observed in brands B, J, H, and I, with average counts of 1.44×10^5 CFU/g, 2.46×10^5 CFU/g, 1.93×10^5 CFU/g, and 1.62×10^5 CFU/g, respectively. Likely, Brands D, E, and F had relatively lower average counts of 3.77×10^4 CFU/g, 4.76×10^4 CFU/g, and 7.92×10^4/g (Table 1).

In general, seasoning packets tend to carry a higher level of microbial contamination than the raw noodles themselves, which might be due to the inclusion of powdered ingredients that were more prone to microbial contamination during processing and packaging. A significant interaction was found between the brand and product type ($F(10, 44) = 21.64$, $p < 0.001$).

Table 1

Total Viable Bacterial Counts in Ready-to-eat Instant Noodle and Their Accompanying Seasoning Samples

S. N.	Brand	Sample	Noodles		Seasonings	
			CFU/gram	Mean CFU/ gram (A1, A2, A3)	CFU/gram	Mean CFU/ gram (A1, A2, A3)
1	A	A1	3.02×10^5	3.03×10^5	1.36×10^6	8.97×10^5
		A2	3.12×10^5		6.57×10^5	
		A3	2.97×10^5		6.726×10^5	
2	B	B1	1.58×10^5	1.70×10^5	1.42×10^6	1.44×10^5
		B2	1.82×10^5		1.50×10^6	
		B3	1.98×10^5			
3	C	C1	2.97×10^5	2.99×10^5	5.1245×10^5	5.71×10^5
		C2	2.92×10^5		6.136×10^5	
		C3	3.07×10^5		5.8815×10^5	
4	D	D1	1.00×10^4	1.00×10^4	3.605×10^4	3.77×10^4
		D2	1.00×10^4		4.6×10^4	
		D3	1.00×10^4		3.105×10^4	
5	E	E1	1.00×10^4	1.00×10^4	5.76×10^4	4.76×10^4
		E2	1.00×10^4		3.75×10^4	
		E3	1.00×10^4		4.77×10^4	
6	F	F1	2.50×10^1	2.50×10^1	7.76×10^4	7.92×10^4
		F2	2.50×10^1		8.745×10^4	
		F3	2.50×10^1		4.77×10^4	
7	G	G1	0.50×10^1	0.50×10^1	1.449×10^5	3.78×10^5
		G2	0		1.0375×10^5	
		G3	0.50×10^1		1.29×10^5	
8	H	H1	1.00×10^1	1.00×10^1	1.9445×10^5	1.93×10^5
		H2	1.00×10^1		2.046×10^5	
		H3	1.00×10^1		1.7935×10^5	
9	I	I1	3.61×104	2.97×10^4	1.992×10^5	1.62×10^5
		I2	5.16×10^4		1.336×10^5	
		I3	1.451×10^4		1.54×10^5	
10	J	J1	8.79×10^4	1.08×10^5	3.764×10^5	2.46×10^5
		J2	1.127×10^5		1.986×10^5	
		J3	1.228×10^5		1.635×10^5	
11	K	K1	3.19×10^4	3.86×10^4	2.48×10^6	1.65×10^6
		K2	3.675×10^4		2.39×10^6	
		K3	4.71×10^4		2.57×10^6	

Pattern of Distribution of Gram-positive and Gram-negative bacteria

A total of 66 samples were examined, including 33 noodle samples and 33 seasoning sachet samples from 11 brands (A, B, C, D, E, F, G, H, I, J, and K). A total of 59 bacterial isolates were obtained, with *Staphylococcus aureus* being the most detected species in both noodle and seasoning sachets. In noodle samples, 8 *Staphylococcus aureus* (brands A, B, D, E, J), 5 *Bacillus* spp. (A, C, J), and eight coagulase-negative *Staphylococcus* (A, B, D, I, J, K) were isolated. Notably, samples F, G, and H showed no detectable bacterial growth in the noodle matrix. Notably, from the noodle sample J, 3 *Klebsiella* spp were isolated, which might be due to contamination during handling or packaging.

Likely, seasoning samples demonstrated the higher microbial load and diversity. In all seasoning samples, 18 *Staphylococcus aureus* was consistently present, highlighting its ubiquitous presence. From the samples of brands, A, C, D, E, F, G, H, and J, 14 *Bacillus* spp. were isolated, and 3 Coagulase-negative *Staphylococci* were obtained from the seasoning samples of Brands A, D, and J (Table 2).

Table 2

Bacterial Types Isolated From Noodle and Seasoning Samples

Samples	Bacterial isolates in noodles	Bacterial isolates in seasonings
A	*Staphylococcus aureus, Bacillus* spp, *CoNS*	*Staphylococcus aureus, Bacillus, CoNS*
B	*Staphylococcus aureus, CoNS*	*Staphylococcus aureus*
C	*Bacillus*	*Staphylococcu aureus, Bacillus*
D	*Staphylococcus aureus, CoNS*	*Staphylococcus aureus, Bacillus, CoNS*
E	*Staphylococcus aureus*	*Staphylococcus aureus, Bacillus*
F	-	*Staphylococcus aureus, Bacillus*
G	-	*Staphylococcus aureus, Bacillus*
H	-	*Staphylococcus aureus, Bacillus*
I	*CoNS*	*Staphylococcus aureus*
J	*Staphylococcus, Bacillus, CoNS, Klebsiella* spp	*Staphylococcus aureus, Bacillus, CoNS,*
K	*CoNS*	*Staphylococcus aureus*

Prevalence of Bacteria Isolated from Noodle and Seasonings

Out of the total of 59 bacterial isolates isolated from various ready-to-eat instant noodle and seasoning samples, *Staphylococcus aureus* were the predominating and frequent bacteria with a total prevalence of 44.1% (26/59), following *Bacillus* spp of 32% (19/59), Coagulase negative *Staphylococcus* of 18.6% (11/59) and *Klebsiella* spp of 5.1% (3/59) (Table 3).

Table 3

Prevalence of Bacteria Isolated From Noodles and its Accompanying Seasonings Samples

Noodle brands	Samples	Isolated Bacteria				Total Percentage
		Staphylococcus aureus	*Bacillus* spp	*CoNS*	*Klebseilla* spp	
A	Noodle	1.7% (1/59)	3.4% (2/59)	3.4% (2/59)		8.5%
	Seasoning	3.4% (2/59)	5.1% (3/59)	1.7% (1/59)		10.2%
B	Noodle	3.4% (2/59)		1.7% (1/59)		5.1%
	Seasoning	5.1% (3/59)				5.1%
C	Noodle		1.7% (1/59)			1.7%
	Seasoning	3.4% (2/59)	1.7% (1/59)			5.1%
D	Noodle	3.4% (2/59)		1.7% (1/59)		5.1%
	Seasoning	3.4% (2/59)	1.7% (1/59)	1.7% (1/59)		6.8%
E	Noodle	3.4% (2/59)				3.4%
	Seasoning	1.7% (1/59)	3.4% (2/59)			5.1%
F	Noodle	0	0	0		0
	Seasoning	1.7% (1/59)	3.4% (2/59)			5.1%
G	Noodle	0	0	0		0
	Seasoning	1.7% (1/59)	3.4% (2/59)			5.1%
H	Noodle	0	0	0		0
	Seasoning	1.7% (1/59)	3.4% (2/59)			5.1%
I	Noodle			1.7% (1/59)		1.7%
	Seasoning	3.4% (2/59)				3.4%
J	Noodle	1.7% (1/59)	3.4% (2/59)	3.4% (2/59)	5.1% (3/59)	13.6%
	Seasoning	1.7% (1/59)	1.7% (1/59)	1.7% (1/59)		5.1%
K	Noodle			1.7% (1/59)		1.7%
	Seasoning	3.4% (2/59)				3.4%
Total		44.1% (26/59)	32.2% (19/59)	18.6% (11/59)	5.1% (3/59)	100%

Antibiotic Susceptibility Pattern of the Isolated Bacteria Isolated from Noodles and Seasonings

The results of the antibiotic susceptibility testing provided important evidence on the effectiveness of common antibiotics against three types of bacterial species isolated from ready-to-eat noodles and their seasonings: *Staphylococcus aureus* (N=26), *Klebsiella* spp. (N=3), and Coagulase-negative *Staphylococci* (CoNS) (N=11). Among the *Staphylococcus aureus* isolates, all (100%) were found to be sensitive to Chloramphenicol, Doxycycline Hydrochloride, Gentamicin, Co-trimoxazole, Azithromycin, and Ciprofloxacin. All the isolates obtained showed complete (100%) resistance to Ampicillin. Similarly, all *Klebsiella* spp. isolates were sensitive to Chloramphenicol, Doxycycline, Gentamicin, Ciprofloxacin, and Co-trimoxazole, and 100% were resistant to Azithromycin and Ampicillin. Coagulase-negative *Staphylococci* also demonstrated 100% sensitivity to all antibiotics tested except for Ampicillin, against which they showed complete resistance (Table 4).

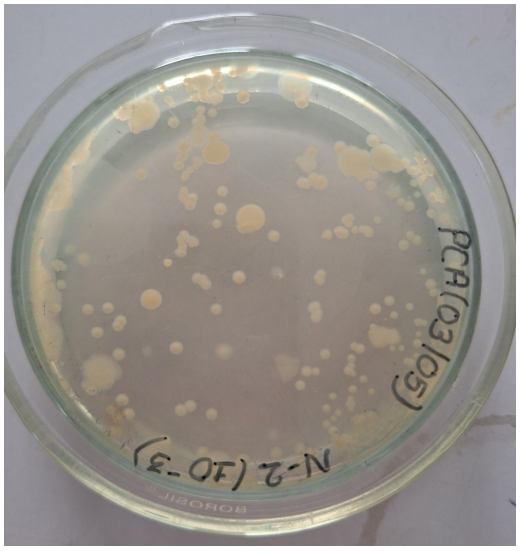
Table 4

Antibiotic susceptibility pattern of isolated bacteria from noodles and seasonings

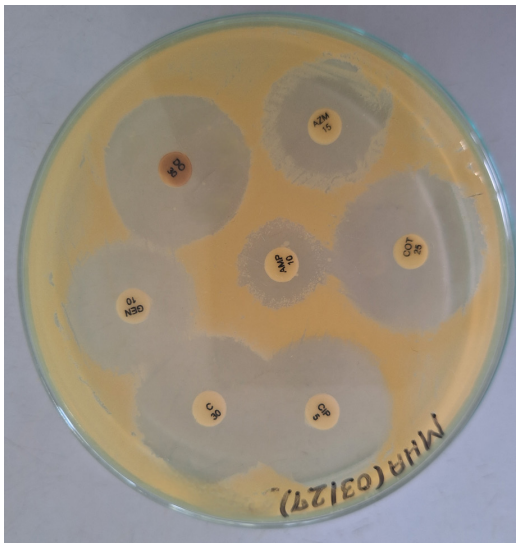
Antibiotics (mcg)	*Staphylococcus aureus* N=26		*Klebseilla* spp N=3		Coagulase-negative *Staphylococci* N=11	
	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive
Chloramphenicol (30)	0	26 (100%)	0	3 (100%)	0	11 (100%)
Doxycycline Hydrochloride (30)	0	26 (100%)	0	3 (100%)	0	11 (100%)
Gentamicin (10)	0	26 (100%)	0	3 (100%)	0	11 (100%)
Co-Trimoxazole (25)	0	26 (100%)	0	3 (100%)	0	11 (100%)
Azithromycin (15)	0	26 (100%)	3 (100%)	0	0	11 (100%)
Ciprofloxacin (5)	0	26 (100%)	0	3 (100%)	0	11 (100%)
Ampicillin (10)	26 (100%)	0	3 (100%)	0	11 (100%)	0

Figure 1 and 2

Culture plates showing (a) Plate count agar (PCA) used to obtain the total microbial count with visible colonies using the spread plate technique and (b) antibiotic sensitivity testing done on Muller-Hinton agar (MHA) with antibiotic discs.



(a)



(b)

The findings of this study highlighted the need for improved hygiene practices and regular monitoring for microbial and antibiotic resistance patterns in ready-to-eat instant noodles particularly the seasoning sachets to protect public health.

DISCUSSION

This study aimed to evaluate the microbiological quality and antibiotic susceptibility profiles of bacterial isolates from ready-to-eat noodles and their corresponding seasoning sachets from 11 brands available in the Pokhara Valley, Nepal. A total of 33 noodles and 33 seasonings (66 samples total) were tested. This study found differences in microbial contamination levels across brands, with some exceeding the acceptable safety threshold, indicating possible deficiencies in product quality control and hygiene throughout the manufacturing and packaging process. Among the tested noodle samples, Brands A and C had the highest microbial counts of 3.03×10^5 CFU/g and 2.99×10^5 CFU/g, respectively. In comparison, Brand B exhibited a moderate level of contamination of 1.70×10^5 CFU/g. The average counts obtained in this investigation exceeded the general acceptable values for acceptable microbiological quality in ready-to-eat or ready-to-cook foods set by the International Commission on Microbiological Specifications for Foods (ICMSF), which suggested a benchmark of dry foods and spices counts $\leq 10^4$ good quality, 10^4 -10^6 CFU/g as marginal, and $> 10^6$ CFU/g unacceptable (ICMSF, 2002). Brands G, F, and H consistently demonstrated very low bacterial counts; Brand G exhibited almost sterile conditions (0.50×10^1 CFU/g), indicative of microbiological control, which may be due to a combination of increased manufacturing standards, packaging, or processing.

The differences found between brands indicate a significant lack of consistency in manufacturers' adherence to food safety practices. Here, noodles from Brands D, E, I, J, and K were contaminated at intermediate levels, and brand J indicated considerable intra-sample variation, with an average count of 1.08×10^5 CFU/g. This variation could indicate inadequate sanitation procedures or quality control measures that failed across production lots, consistent with previous studies that described a risk of microbial contamination in ready-to-eat foods from developing markets (Mensah et al., 2012; Tambekar et al., 2009). Most importantly, the data supported the notion that failure to implement Good Manufacturing Practices (GMP), or failures in Hazard Analysis and

Critical Control Points (HACCP), may contribute to contamination in processed food items.

Seasoning sachets showed higher bacterial loads than noodles. Brand K had the highest contamination (average count of 1.65×10^6 CFU/g), followed by Brands A and B at 8.97×10^5 and 1.44×10^5 CFU/g, respectively. Brands C, G, J, H, I, and J also had elevated levels in the 10^5 CFU/g range, while loads for brands D, E, and F were lower but still noteworthy. These results underscored the concern that seasoning sachets could be contaminated with microorganisms, as the powdered ingredients were not necessarily sterilized. These findings aligned with other studies, which demonstrated that powdered ingredients such as spices, flavour enhancers, and dehydrated vegetables were common in seasoning sachets and could be a source of microorganisms if not irradiated or heat-treated (Van Doren et al., 2013; Banerjee & Sarkar, 2003). Additional microbial contamination might occur during post-process handling, packaging, or storage, especially for products that did not meet sufficient hygiene standards. Past studies reported these conditions, in which spices and dry seasonings contained higher-than-acceptable aerobic bacterial counts and some pathogenic bacteria due to inadequate sanitation during processing (Budathoki et al., 2021; Nguyen et al., 2021).

The finding that seasoning packets consistently had higher bacterial loads than the noodle samples reflected a unique weakness in the handling and processing of dry powder ingredients. As noted above, noodles often remained free of harmful microorganisms, which might be due to exposure to a lethal process such as steaming, frying, or baking. In contrast, seasonings might skip this step and rely solely on packaging to ensure their safety for use. The lack of microbial hazard control means the consumer's health remains at risk when seasoning packets are added after limited cook time or when product instructions do not indicate a full boil. The fact that seasonings had bacterial counts significantly higher than those of the noodles, especially those exceeding 10^6 CFU/g, indicated possible spoilage or exposure to pathogenic organisms, such as *Bacillus cereus*, *Salmonella* spp, or *Staphylococcus aureus*, that notoriously survive dry food matrices (Beuchat et al., 2013; Siti et al., 2020).

Gram-positive bacteria were predominant (94.92%) among 59 total isolates. *Staphylococcus aureus* was the most commonly isolated organism (44.1%), which was consistent with findings of earlier studies that also identified *S. aureus* as a frequent contamination in ready-to-eat foods due to improper handling or post-processing (Bennett et al., 2013; Argudin et al., 2010). 32.2% *of Bacillus* spp were also observed in both noodles and seasonings, which could survive standard cooking processes and generate toxins, especially by *Bacillus cereus,* which can cause food poisoning (Lucking et al., 2013; Ehling-Schulz et al., 2019). Although they were commensal, coagulase-negative *Staphylococcus* accounted for 18.6%, and could act as reservoirs for antibiotic resistance (Becker et al., 2014).

The presence of Gram-negative bacteria was much lower where *Klebsiella spp* were found 5.1% only in the noodle sample of brand J which might be due to fecal or environmental contamination during post-cooking packaging or using contaminated water in food processing which could serve as a potential vector for enteric bacteria and raised the public health concern since being opportunistic pathogens, these bacteria were often linked to nosocomial and community acquired infections (Podschun & Ullmann, 1998; Srivastava et al., 2022). Notably, samples F, G, and H did not show growth of any bacterial isolates, indicating the good hygienic controls during manufacturing and packaging processes.

The bacterial isolates of *Staphylococcus aureus*, Coagulase negative *Staphylococcus and Klebsiella* spp were sensitive to the tested antibiotics chloramphenicol, doxycycline, gentamicin, ciprofloxacin, and co-trimoxazole but they were resistant to ampicillin. Similarly, *Klebsiella* spp showed resistance to ampicillin and azithromycin, findings supported by a study by Okonko et al. (2011), who also demonstrated multidrug-resistant *Klebsiella pneumoniae* from food and water sources in Nigeria. Beta-lactam antibiotics such as penicillin and ampicillin-resistant *Staphylococcus aureus* strains have been reported worldwide in food isolates (Normanno et al., 2007).

The resistance to ampicillin observed among the isolates was likely due to the extensive use of this antibiotic in both human and veterinary medicine, which has selected bacteria that produce beta-lactamases capable of inactivating the drug (Normanno et al., 2007; Budathoki et al., 2021). The susceptibility of all isolates to Co-Trimoxazole, Gentamicin, Doxycycline, and Chloramphenicol indicated the potential for these antibiotics to be prescribed effectively for infections caused by bacteria from similar sources of transmission. It was critical to monitor bacterial resistance to inform the appropriate use of antimicrobials. Resistant bacteria in instant noodles and seasonings were alarming, as they could act as vehicles for transferring antimicrobial resistance through the food chain. *S. aureus* can produce heat-stable enterotoxins which are not destroyed when cooked and may cause food poisoning (Argudin et al., 2010). Similarly, *Bacillus cereus* is also capable of causing food poisoning if food is kept in room temperature for longer duration (Wang et al., 2022). Although its presence was very less common in food, *Klebsiella* spp is a pathogen of concern in food due to its capacity to cause respiratory or urinary tract infections (Podschun & Ullmann, 1998).

The instant noodles and seasoning sachets showing high bacterial contamination rates and antibiotic-resistant isolates in this study reflected the limited regulatory supervision, insufficient hygiene practices during manufacturing, packing, and storage, and the limited microbiological monitoring of RTE instant noodles in Nepal. These findings highlighted the pressing requirement for the fortification of food safety laws, routine microbial testing, and public health measures to reduce contamination risks, and to prevent the dissemination of antimicrobial-resistant bacteria in the most consumed convenience foods like instant noodles.

CONCLUSION AND RECOMMENDATIONS

The results showed considerable amounts of microbial load and antibiotic-resistant bacteria in ready-to-eat instant noodles highlighting the necessity of close food safety monitoring, improved hygiene during production and packaging and better education about the health risks associated with contaminated ready-to-eat instant noodles. Local government should consider mandatory testing for bacteria in food and establish hygiene standards for the proper handling and packaging of food as a way to reduce contamination and curb the spread of antimicrobial resistance. Such policy actions are important for consumer safety as well as strengthening governance of food safety in the context of Nepal.

This study was conducted focusing on bacterial contamination, in this regard future researches could evaluate the possibility of fungal contamination in instant noodle products. Further studies with larger sample sizes, multicentric and molecular assessments would help with an overall better interpretation of microbial contamination and resistance of resistance patterns to ready-to-eat foods like instant noodles.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENTS

We thankfully acknowledge the Centre for Research and Innovation (CRI) , Prithvi Narayan Campus, Pokhara, Nepal to make this study possible by providing the mini research grant.

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