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Biochemical Characterization and Antimicrobial Susceptibility Test of the Bacterial Strain Isolated from Sandwich in Rajshahi University, Bangladesh

Rubait Hasan[1]*, Jamiatul Husna[1], Mohammad Shahangir Biswas[1], Foyzur Rahman[1], Md. Faruk Hossain[1], Shahidur Rahman[1], Munna Kumar Podder[1], and Mohammad Zakerin Abedin[2]

[1]Dept. of Department of Biochemistry and Biotechnology, School of Biomedical Sciences, Khwaja Yunus Ali University, Sirajgonj-6751, Bangladesh; and [2]Dept. of Microbiology, School of Biomedical Sciences, Khwaja Yunus Ali University, Sirajgonj-6751, Bangladesh.

*Correspondence: hasanru88@gmail.com (Rubait Hasan, Lecturer, Department of Biochemistry and Biotechnology, Khwaja Yunus Ali University, Sirajgonj-6751, Bangladesh).

ABSTRACT

This study was conducted to isolate and observe the morphological and biochemical characteristics of bacterial strains present in the sandwich. A single bacterial colony was isolated from a sandwich collected from different restaurants located in the area of the University of Rajshahi by plating from the diluted primary bacterial suspension of the liquid medium onto an agar solidified mineral salt medium after purifying through filter paper. The isolated bacterium was found to be Gram-positive, coccus, motile, lactose-non-fermenting, and could utilize different carbohydrates. Bacterial strain A showed a positive result for the Methyl Red test, the Catalase test, the Indole test, and the Simmons citrate agar test. The optimum culture condition of the isolate was pH 8 and the salt concentration was 0.1 gm/100 ml. The Minimum Inhibitory Concentration (MIC) value against Vancomycin was 50mg/ml and the viable cell count indicated 459×10^7 CFU/ml. The result showed that the isolated bacterial strain A was resistant to Vancomycin and amoxicillin, whereas it was susceptible to gentamycin, ciprofloxacin, and chloramphenicol. This bacterial strain A can grow to a harmful extent after a certain time of incubation, which may cause a health hazard.

Keywords: Isolation, Characterization, Sandwich, Antibiotic susceptibility, MIC, and Rajshahi university.

INTRODUCTION:

Food is a chemically complex matrix that contains sufficient nutrients to support microbial growth. Several factors, such as water availability, pH, and temperature, encourage, prevent, or limit the growth of microorganisms in foods (Sahu and Bala, 2017, Bradford *et al.*, 2018). Fast food includes different food items like pizza, burgers, or French fries, and Chinese as well as Indian (Pérez-Hernández, 2019; Happy *et al.,* 2018; and Schoffman *et al.,* 2016).

A sandwich is a food item consisting of one or more types of food placed on or between slice of bread, or more generally, any dish wherein two or more pieces of bread serve as a container or wrapper for some other food (Semlak, 2011). The sandwich was originally a portable food item or finger food, which began its popularity in the Western World. Today, sandwiches in various versions are found worldwide. Microbial food safety is an increasing public health concern worldwide. Each year in the United States, it is esti-

mated that approximately 76 million food-borne illnesses are caused by *Campylobacter spp*., nontyphoidal *Salmonella*, and pathogenic *Escherichia coli*, all of which colonize the gastrointestinal tracts of a wide variety of wild and domestic animals, particularly those raised for human consumption (Meng and Doyle, 1998). Contaminated raw or undercooked poultry and red meats are particularly important in transmitting these food-borne pathogens.

The consumption of fast foods, raw milk, and raw milk products has been reported to be associated with serious health problems (Fusco *et al.,* 2020; Wang *et al.,* 2018, Popkin and Reardon, 2018; Christian *et al*., 2018). Microorganisms in fast and traditional fast foods are responsible for many human diseases, e.g. *Salmonella* bacteria is a common cause of food-borne illness, particularly in undercooked chicken and chicken eggs (Angelillo *et al.,* 2000).

Food-borne diseases are an important cause of morbidity and mortality worldwide. It is estimated that food-borne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year (Mead *et al.,* 1999). An outbreak of listeriosis among hospital patients in 2004 in Wales, the United Kingdom, was epidemiologically linked to the consumption of contaminated sandwiches. Harakeh *et al.* (2005) reported the isolation of *Salmonella* and *E. coli* isolates from meat-based fast food in Lebanon. Food contamination with antibiotic-resistant bacteria can be a major threat to public health as the antibiotic resistance determinants can be transferred to other pathogenic bacteria, potentially compromising the treatment of severe bacterial infections (Kubota *et al.,* 2008). Many reports show the prevalence of antimicrobial resistance among food-borne pathogens (Korkeala and Lindstrom, 2009).

The prevalence of antimicrobial resistance among food borne pathogens has increased during recent decades. There is no available data about fast food microbial existence in the Rajshah University area. Therefore, this study was designed to isolate and subsequently characterize new bacterial strains from sandwiches and their microbial resistance profiles in Rajshahi University, Bangladesh.

MATERIALS AND METHODS:

Collection and Pure Culture of Isolates

Bacteria from sandwiches were isolated by plating from the diluted primary bacterial suspension of the liquid medium onto an agar solidified mineral salt medium. Before plating, sandwiches were collected from different restaurants located in the area of the University of Rajshahi, Bangladesh. Then the collected samples were diluted in autoclaved distilled water and filtered. A filtered solution was added to the Luria-Bertani broth medium and incubated for the growth of bacteria. After incubation, this bacterial suspension was used for plating and single bacterial colony isolation. The prepared plates were incubated at 37°C for overnight and bacterial colonies were found to grow on the medium with different colors.

Microscopic Study of Bacterial Isolates

Gram's staining & Culture media dependent characteristics

Gram's staining was performed as per procedures described by Merchant and Packer, (1969) to differentiate bacterial species into two large groups (Gram-positive and Gram-negative) based on the physical properties of their cell walls (**Fig. 2**). Culture media dependent characteristics were also observed to determine the size, shape, and arrangement of bacteria (**Table 1**).

Motility test

The simplest method to examine living microorganisms and their motility is the hanging drop method (Mandri *et al.,* 2007). The organism is observed in a drop suspended under a cover glass in a concave slide using this method, which is typically observed in a bright field microscope. Cover slides and cover slips are washed with distilled water, dried and wiped with ethanol.

Vaseline is placed on the four edges of the cover of the cover slip. A drop of culture was placed in the center of the cover slip. The cavity slide was placed over the cover slip, and Vaseline was used to make contact between the cover slip and slide. The curve was such that the drop should not touch the inner end of the cavity. The slide was turned upright and observed under a light microscope (**Fig. 3**).

Biochemical Tests

Methyl Red Test

A single colony from the pure culture of the test organism was inoculated in 5 ml of sterile MR-VP broth. After five days of incubation at 37ºC, five drops of methyl red solution were added and observed for color formation. The development of a red or yellow color indicated positive or negative results, respectively (Cheesbrough, 1985).

Catalase Test

A volume of 3 ml of catalase reagent (3% H_2O_2) was taken in a test tube. Isolate A was taken with a glass rod and merged in the reagent and observed for bubble formation, which indicated a positive test. The absence of bubble formation indicated a negative result (Cheesbrough, 1985).

Triple Sugar Iron (TSI) Test

Gram-negative enteric bacilli are identified using triple sugar iron agar based on the generation of hydrogen sulfide and the fermentation of dextrose, lactose, and sucrose. Acids of different colors are produced by organisms that ferment dextrose monohydrate, changing the medium's color from red to yellow (Eaton *et. al*., 2005).

Indole Test

The test organisms were raised in 3 milliliters of peptone water that contained tryptophan at 37ºC for 48 h. One milliliter of diethyl ether was added, shaken vigorously, and left to stand until the ether rose to the top. Then 0.5 ml of Kovac's reagent was gently run down the side of the test tube to form a ring in between the medium and the ether. The development of a brilliant red-colored ring indicated a positive test (Cheesbrough, 1985).

Lactose Fermentation Test

A lactose fermentation test was used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting Gram-negative bacteria. This test was performed by MacConkey (1905).

Citrate Test

The citrate test determines if a bacterial isolate can use citrate as a source of carbon and energy (Difco, 1998). The citrate test is based on the generation of alkaline by-products of citrate metabolism and a subsequent increase in the pH of the medium, which is demonstrated by the color change of a pH indicator from green to blue. Bromothymol blue is the pH indicator. The Gram-negative pathogens and environmental isolates are recognized using the citrate test (Tang *et al*., 2000). The overall results of the biochemical tests of the isolated bacterial strains are summarized in (**Table 4**).

Effect of pH Variations on Growth

The medium was maintained at different pH ranges from 5-8 and bacterial strains were inoculated in differrent pH media and incubated on an orbital shaker at 160 rpm for 36 hrs. It is possible to observe optical density by using a spectrophotometer (**Table 3**).

Effect of Temperatures on Growth

Firstly, the LB media were prepared and the pH was adjusted at 7 for Isolate A. Then the bacterial strain was inoculated in an already prepared LB medium and incubated at ranging from 20^0 C to 35^0C up to 36hrs. The growth rates were observed at different times by using the spectrophotometer (**Table 4**).

Carbohydrate Utilization Test

To find out the ability of the isolates to utilize different carbohydrates, the cultures were inoculated in an MS medium containing different carbohydrates viz. glucose, mannose, arabinose, sucrose, lactose, & cellulose. The final concentration of carbohydrates is 1%. The tubes were incubated at 28^0 C for 3-5 days and observed for any growth (**Table 5**).

Viable Cell Count

Aliquots (2.5 ml) of 24 hr old LB medium-grown bacterial cultures were inoculated into 100 ml Erlenmeyer flasks containing 25 ml of Luria-Bertani broth. Control was maintained with an equal volume of broth without bacterial culture. Viable cell counts were per-formed 24 hours after inoculation to monitor bacterial growth. At regular intervals, a bacterial inoculum (1 ml) was drawn from the test and control cultures, and serial dilutions were performed using 9 ml of sterile saline (0.85% NaCl; pH 8.5). Appropriate dilutions were plated in triplicate on nutrient agar and the plates were incubated at 37^0 C for 24 h (**Table 6**).

Antibiotic Sensitivity Test

Nutrient agar plates were dried at 30º C and overnight grown bacterial culture (OD = 0.5) was poured onto the nutrient plate and dried. Antibiotic disks were pla-

ced centrally on the respective plates and incubated at 30° C (**Table 2**). After overnight incubation, the zone was observed on the plate and measured with the help of an mm scale (**Table 7**).

Minimum Inhibitory Concentration (MIC) Tests

The MIC of antibiotics, Vancomycin, was determined by the turbid metric method against isolated bacteria through the broth tube dilution method. The antibiotics and Vancomycin in various concentrations were applied to the LB broth media in each test tube and incubated at 37°C for 48 hours. The results of the MIC value of Vancomycin against isolated bacteria are presented in **Table 8**.

RESULTS AND DISCUSSIONS:

The food bacteria of greatest importance to human pathology are the most common causes of human infection and are extensively widespread in the environment thanks to fast foods (Kay *et al.,* 1994). The study was conducted to isolate and observe morphological and biochemical characteristics of bacterial strains available in local sandwiches collected from the Rajshahi University Campus Area. A single bacterial colony was isolated from the sandwich (**Fig.1**), and the isolated strain A was subjected to morphological and biochemical tests where the optimum growth conditions at different pH and salt concentrations were observed. Morphological and biochemical characteristics of microorganisms are important tools for identification and observation of their distinguishable features. The interplay of factors affecting microbial growth in foods, such as (water activity, pH, and temperature) ultimately determines whether micro-organisms will grow in a given food. Morphological and biochemical tests were performed and the isolated bacterium was found to be gram negative, coccus, motile, lactose non-fermenting, and had the ability to utilize different carbohydrates. Bacterial strain a showed positive results for the Methyl Red test, Catalase test, Indole test, and Simmons citrate agar test. The optimum culture condition of the isolate was at pH 7 (**Fig. 4**) and the salt concentration was 0.1 gm/100 ml. Juneja *et al.* (2002) demonstrated the presence of microorganisms, e.g., *Salmonella sp, Staphyloccus aureus,* and *Bacillus species* in minimally processed bakery products. Similarly, from bread and bakery products, *Bacillus strains* were isolated (Collins *et al*., 1991). As the certain number of viable cells of bacteria is associated with food spoilage and health hazard, viable cell count was conducted at specific media to reveal the extent of the growth of the isolate. Viable cell count indicated 459×10^7 CFU/ml. The high microbiological contamination could be due to post-contamination between preparation and consumption. Coliforms and *E. coli* may contaminate sandwiches during processing through contamination and faecal material as a result of poor sanitary practices, improper handling, and improper hygiene conditions (Bostan *et al*., 2005). The presence of multidrug-resistant strains is alarming because such strains lead to a higher fatality rate than sensitive ones. In this research work, the antibiotic sensitivity test was conducted to reveal the resistance and susceptibility pattern of the isolated bacterium. The resistant pattern of the isolated strain A was tested against Amoxicillin, Penicillin, Erythromycin, Vancomycin, Gentamicin, Ciprofloxacin, and chloramphenicol. The isolate showed resistance to Amoxicillin, Penicillin, & Vancomycin and was found to be susceptible to Erythromycin, Gentamycin, Ciprofloxacin, and chloramphenicol. In our experiment, 18 mm, 14 mm, 21 mm, 11 mm, and 2 mm zones of inhibition were found for chloramphenicol, Ciprofloxacin, Gentamycin, Erythromycin, and Amoxicillin (**Fig. 6**).

The Minimum Inhibitory Concentration (MIC) value against Vancomycin was found to be 50mg/ml for the strain, demonstrating that a low concentration of this antibiotic was required to inhibit the growth of this microorganism. For bacterial growth in bakery foods, storage duration plays an important and vital role, and long storage duration favors more bacterial growth, so always try to avoid fast food storage for a long time. As most bacteria are able to produce toxins, it is recommended for strict monitoring and certification of bakery foods, hoping to maintain the quality of bakery foods and ultimately to ensure good health. The study was confined only to certain shops located in the area of the University of Rajshahi, Bangladesh, so the result does not represent the whole country. Detailed study is required concerning more areas, increasing sampling sites and their numbers. Further work is necessary to identify this strain, and 16S rDNA sequencing is needed to confirm the species of the strain.

Table 1: The isolated bacterial strain A's culture media-dependent characteristics and microscopic observation.

Agar media	Characters	Results (Strain A)
Mineral salts agar media	Size	(1-2) mm
	Shape	Round
	Color	Cream
	Consistency	Sticky
	Opacity	Translucent
	Elevation	Raised
	Margin	Entire
Nutrient agar media	Abundance of growth	Good
	Color	Cream
Nutrient broth media	Turbidity	Uniform with fine turbidity
Microscopic observation	Gram staining	Gram-negative
	Motility	Motile

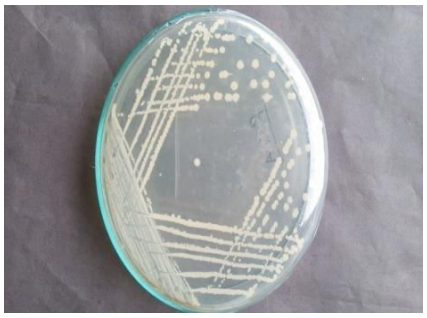

Fig. 1: Isolated bacterial colonies.

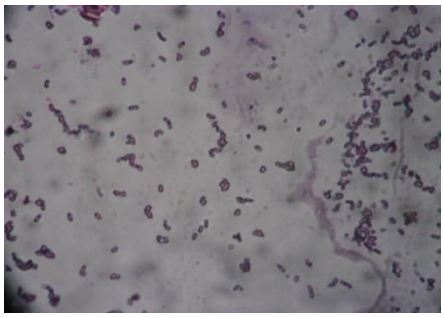

Fig. 2: Microscopic view of isolated a strain.

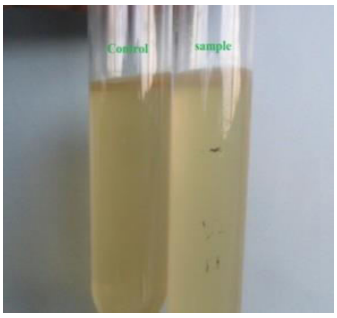

Fig. 3: Motility test.

Table 2: Effects of pH on the growth of isolated bacterial strain A.

Time (hr)	Optical Density (OD) at 620 nm				
	pH 5.0	pH 6.0	pH 7.0	pH 8.0	Control
0	0.03	0.04	0.06	0.05	0.05
2	0.03	0.045	0.065	0.05	0.05
4	0.13	0.16	0.17	0.17	0.05
6	0.23	0.24	0.30	0.25	0.05
8	0.25	0.27	0.40	0.32	0.05
10	0.27	0.30	0.45	0.35	0.05
12	0.30	0.35	0.50	0.40	0.05
24	0.40	0.49	0.60	0.50	0.05
36	0.38	0.32	0.39	0.45	0.05

Table 3: Effects of temperature on the growth of bacterial strain A.

Time (hr)	Optical Density (OD) at 620 nm				
	20^0 C	25^0 C	30^0 C	35^0 C	Control
0	0.03	0.04	0.06	0.05	0.05
2	0.03	0.045	0.065	0.05	0.05
4	0.13	0.16	0.17	0.17	0.05
6	0.23	0.24	0.30	0.25	0.05
8	0.25	0.27	0.40	0.32	0.05
10	0.27	0.30	0.45	0.35	0.05
12	0.30	0.35	0.50	0.40	0.05
24	0.40	0.49	0.60	0.50	0.05
36	0.38	0.32	0.39	0.45	0.05

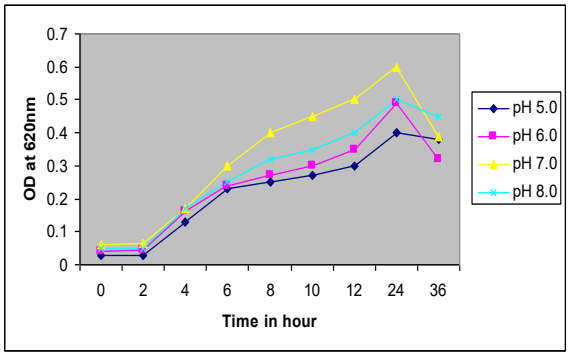

Fig. 4: Effect of pH on Bacterial Growth

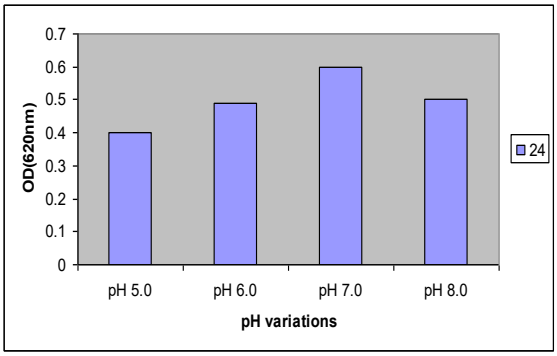

Fig. 5: Effect of temperature on Bacterial Growth

Table 4: Biochemical test of bacterial strain A.

Characters	Results
Methyl Red (MR)	Positive
Catalase test	Positive
Sulphide test	Negative
Indole test	Positive
Citrate agar test	Positive
Lactose fermentation test	Negative

Table 5: Utilization of carbon sources by the strain A.

Carbon sources	Utilization
Arabinose	Positive
Galactose	Negative
Sucrose	Positive
Maltose	Positive
Fructose	Positive
Lactose	Negative
Cellulose	Negative
Xylose	Negative

Table 6: Detection of viable cells.

Time (hour)	OD (nm)	Culture media	CFU/ml	Control
24hr	620	Luria-Bertani (LB)	459×10^{7}	0

Table 7: Antibiotic sensitivity test.

Antibiotics (µg/disc)	Zone of Inhibition	R	S and I
Amoxicillin (**10µg**)	2mm	R	-
Penicillin (**10µg**)	0mm	R	-
Erythromycin (15µg)	11mm	-	I
Vancomycin(**30µg**)	0mm	R	-
Gentamycin(10µg)	21mm	-	S
Ciprofloxacin (5µg)	14mm	-	S
Chloramphenicol (30µg)	18mm	-	S

Here, (5~10mm) = Resistance to antibiotic (R), (10^{+}~15mm) = intermediate resistance (I), (15^{+}~20mm) = Sensitive to antibiotic(S).

Fig. 6: Antibiotic sensitivity test.

Here, A = Amoxicillin, Ci = Ciprofloxacin, Ch= Chloramphenicol, E = Erythromycin, G = Gentamycin, P= Penicillin, V = Vancomycin

Table 8: Minimum Inhibitory Concentration of Vancomycin.

Test organism	Concentration	Growth
Bacterial Strain A	100	-
	50	-
	25	+
	12.5	+
	6.25	+
	3.125	+
	1.5625	+

The '+' sign indicates the growth of the microorganisms while '-' sign indicates no growth.

CONCLUSION:

The bacteriological condition of the samples and their safety assessment revealed that the food contained some degree of bacteria. From this study, it was concluded that our isolated bacteria were gram-negative and motile with positive results for various tests: methyl red, catalase indole, and citrate agar. The bacterial isolate showed the optimum pH and temperature for growth was 7.0 and 37°C respectively. The isolate showed resistance to Amoxicillin, Penicillin, and Vancomycin and was observed to be susceptible to Erythromycin, Gentamycin, Ciprofloxacin, and Chloramphenicol. The Minimum Inhibitory Concentration (MIC) value against Vancomycin was found to be 50mg/ml for the strain A. The microbial safety of the investigated sandwich depends not only on the environmental conditions but also on personal hygiene. The future prospects are of identifying this strain using 16S rDNA sequencing to confirm the species of the strain.

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CONFLICTS OF INTEREST:

The authors declare there is no with conflicts with due respect to the publication of this article.

REFERENCES:

1) Angelillo, I.F., Rizzo, L. and Bianco, A., (2000). Food handlers and foodborne diseases: knowledge, attitudes, and reported behavior in Italy. *Journal of food protection*, **63**(3), pp.381-385. https://doi.org/10.4315/0362-028x-63.3.381
2) Bostan, K., Cetin, O. and Ergun, O., (2005). The presence of verotoxinogenic *E. coli* in some foods. *Veterinarskiglasnik*, **59**(3-4), pp.427-436.
3) Bradford, K. J., Dahal, P., & Wu, F. (2018). The dry chain: Reducing postharvest losses and improving food safety in humid climates. *Trends in Food Science & Technol.*, **71**, 84-93. https://doi.org/10.1016/j.tifs.2017.11.002
4) Cheesbrough, M., (1981). Medical laboratory manual for tropical countries, **1**, M. Chees-brough, 14 Bevills Close, Doddington, Cambridgeshire, PE15 OTT.
5) Christian, M. and Gereffi, G., (2018). Fast-food value chains and childhood obesity: A global perspective. In Pediatric obesity, *Humana Press, Cham*. pp. 717-730. https://www.majesticgrades.com/fast-food-value-chains-and-childhood-obesity/
6) Difco, (1998). Difco Manual, Detroit, MI 11th edition.
7) Fusco, V., Chieffi, D., Böhnlein, C. and Franz, C.M., (2020). Microbial quality and safety of milk & milk products in the 21st century. *Comprehensive Reviews in Food Science and Food Safety*, **19**(4), pp.2013-2049. https://doi.org/10.1111/1541-4337.12568
8) Happy A. H., Alam M. G., Mahmud S., Uddin M. E. (2018). Isolation, identification & characterization of gram-negative bacteria from popular street food (Chotpoti) at Savar area, Dhaka, Bangladesh, *Open Access Library J*. **5**, e4986. https://doi.org/10.4236/oalib.1104986
9) Harakeh, S., Toufeili, I. and Tannous, R., (2005). Isolation, molecular characterization and antimicrobial resistance patterns of Salmonella and Escherichia coli isolates from meat-based fast food in Lebanon. *Science of the Total Environment*, **341**(1-3), pp.33-44.
10) Juneja, V. K., Novak, J. S. and Sapers, G. M., (2002). Microbial safety of minimally processed foods.*CRC press*. https://doi.org/10.1201/9781420031850
11) Kalantari, S., Sepehri, G., Bahrampour, A. and Sepehri, E., (2012). Determination of bacterial

contamination isolated from Sandwiches in Kerman City and their resistance to commonly used antimicrobials. *Archives of Applied Science Research*, **4**(2), pp.1100-1105.

12) Kay, B.A., Griffin, P.M., Strockbine, N.A. and Wells, J.G., (1994). Too fast food: Bloody diarrhea and death from *Escherichia coli* 0157: H7. *Clinical Microbiol Newsletter*, **16**(3), pp.17-19.
13) Korkeala, H. and Lindström, M., (2009). Foodborne bacterial diseases. Duodecim; *LaaketieteellinenAikakauskirja*, **125**(6), pp.674-683.
14) Kubota, K., Angulo, F.J. and Morikawa, K., (2008). The human health burden of foodborne infections caused by *Campylobacter, Salmonella*, and *Vibrio parahaemolyticus* in Miyagi Prefecture, Japan. *Food borne Pathogens and Disease,* **5**(5), pp.641-648. https://doi.org/10.5851/kosfa.2015.35.5.674
15) Li, N., Chen, W., Zheng, H. and Liu, Z., (2018). Variation in raw milk microbiota throughout 12 months and the impact of weather conditions. *Scientific reports*, **8**(1), pp.1-10.
16) Mandri, T. and Lin, J., 2007. Isolation and characterization of engine oil degrading indigenous microorganisms in Kwazulu Natal, South Africa *African journal of Biotechnology*, **6**(1). https://doi.org/10.4236/oalib.1104016
17) MacConkey, A., (1905). Lactose-fermenting bacteria in faces. *Epidemiology and Infection,* **5**(3), pp.333-379.
18) Mead, P.S., Slutsker, L., and Tauxe, R.V., (1999). Food-related illness and death in the United States. *Emerging infectious diseases*, **5**(5), p.607-625. https://doi.org/10.3201/eid0505.990502
19) Meng, J. and Doyle, M.P., (1998). Emerging and evolving microbial foodborne pathogens. *Bulletin de l'Institut Pasteur,* **96**(3), pp.151-163.
20) Merchant IA, Packer RA, (1969). Veterinary Bacteriology and Virology. 7th ed., The Iowa *State University Press*, *Ames, Iowa, USA*. pp. 211-305.
21) Pérez-Hernández, L., (2019). XL burgers, shiny pizzas, and ascending drinks: Primary metaphors and conceptual interaction in fast food printed advertising. *Cognitive Linguistics*, **30**(3), pp.531-570. https://doi.org/10.1515/cog-2018-0014
22) Popkin, B.M. and Reardon, T., (2018). Obesity and the food system transformation in Latin America. *Obesity Reviews*, **19**(8), pp.1028-1064.
23) Sahu, M. and Bala, S., (2017). Food processing, food spoilage and their prevention: An overview. *Int. J. Life. Sci. Scienti. Res*, **3**(1), pp.753-759.
24) Schoffman, D.E., Davidson, C.R., and Turner-McGrievy, G.M., (2016). The fast-casual conundrum: fast-casual restaurant entrees are higher in calories than fast food. *Journal of the Academy of Nutrition & Dietetics*, **116**(10), pp.1606-1612.
25) Semlak, J.L. and Pearson, J.C., (2011). Big Macs/Peanut Butter and Jelly: An exploration of dialectical contradictions experienced by the sandwich generation. *Communication Research Reports*, **28**(4), pp.296-307. https://doi.org/10.1080/08824096.2011.616449

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