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TEST OF TOTAL PLATE COUNT AND BACTERIA CHARACTERISTICS IN ENDO AGAR IN REFILLABLE DRINKING WATER AT DEPOT, CILEUNGSI DISTRICT, BOGOR

Marsheila Salsabila Lazuardiyan[1*], Noor Andryan Ilsan[1], Maulin Inggraini[1]

Abstract

Introduction: Refill Drinking Water Depot (DAMIU) is a business entity that processes raw water into drinking water. The quality requirements for drinking water quality are regulated in the Minister of Health Regulation Number 492/MENKES/PER/IV/2010 based on the bacteriological requirements of drinking water, namely 0/100 ml of water and the quality standards for demineralized water or refillable drinking water which are regulated in the Indonesian National Standard (SNI 6241:2015) which is 1,0 x 10^2 CFU.

Method: The design of this research is descriptive. Samples were planted using the spread plate method after starified dilution. The object of the research is refill drinking water taken from refill drinking water depot along Cileungsi District, Bogor Regency with a population of 10 samples and taken using a non-probability method with purposive sampling technique. Data analysis using SPSS V.25 software with One Way ANOVA (Analysis of Variance) statistical test.

Results: The result of the One Way ANOVA statistical test obtained a p value of 0.01 (<0.05), which indicates that there is a significant difference in the amount of growth between samples of refill drinking water at depot.

Conclusion: Based on SNI 6241:2015, the results of the TPC calculation showed that 3/10 DAMIU (30%) be found the bacteriological requirements of drinking water, namely in DAM 3 of 0.5 x 10^2 CFU/ml, DAM 5 of 0.4 x 10^2 CFU/ml, and DAM 6 of 0.4 x 10^2 CFU/ml. Pathogenic bacteria suspected of contaminating refilled drinking water based on the characterization of Endo Agar and biochemical test media in the form of Simmon Citrate Agar, D- Glucose Broth and Lactose Broth tests are *Escherichia coli*, *Klebsiella sp*, and *Pseudomonas aeruginosa*.

Key words *: Brain Heart Infusion Agar (BHI-A), Coliform, Endo Agar, Escherichia coli, One Way ANOVA Test, Refill drinking water, Total Plate Count Method (TPC)*

INTRODUCTION

The high demand for drinking water encourages consumers to use find an alternative to found the needs of water in the body. Water Packaged Drinking (AMDK) is the first alternative to fulfill drinking water needs, for reasons that are more practical and hygienic. But over time the price of bottled water with various brands available to more expensive. Therefore, consumers turn to the second alternative, which is by consume a drinking water produced by Refill Drinking Water Depot (DAMIU). DAMIU is included in the business that processes treatment of raw water for drinking water. When compared in terms of price between AMDK and DAMIU of course refill water is cheaper. But Although the price is relatively affordable, not all drinking water depots are filled has a hygienic quality that is well maintained (Mila, Nabilah, and Puspikawati, 2020).

According to Permenkes No. 492/MENKES/PER/IV/2010 based on the bacteriological requirements of drinking water, which is not allowed to contain disease bacteria that exceeds the predetermined threshold, which is 0/100 ml of water, while the quality standards for demineralized water or refilled drinking water are regulated in the Standard National Indonesian (SNI 6241:2015) which is 1.0 x 10^2 CFU. According to data from the Bogor City Health Office, in 2017 bacteriological tests were carried out on 61 DAMIU. The test results from the 61 depots, 31 depots contained pathogenic bacteria that is *Escherichia coli*.

The presence of pathogenic bacteria such as *E. coli* that exceeds the threshold can cause health impacts for humans, one of which is diarrhea. As for the prevalence of diarrhea, according to the Profile of the Health Office of the City of Bogor, there has been an increase in cases over the last 2 years. In 2019 in Bogor City there were 18,492 cases of diarrhea, while cases of diarrhea that occurred in 2020 increased by

18,751. Diarrhea is a common condition that results from reduced absorption of water by the intestines or usually an increase in water secretion which is described as loose, watery stools that occur three or more times a day. Diarrhea is grouped into three categories; watery, fatty (malabsorption), and infectious. In the case of watery diarrhea is usually caused by bacteria. One of the pathogenic bacteria that attacks is *E. coli* which causes injury to the intestinal epithelium so that it is unable to absorb water and causes the feces to become watery (Valerie and Nicholas, 2017).

Based on the impacts and problems that occur in the community, a study related to counting the number was carried out to see the characteristics of pathogenic bacteria in drinking water depots. Previous research by Rahayu and Kusmawati (2018) regarding *E.coli* contamination in complementary drinking water from drinking water reservoirs in Malang City *E.coli* contamination was found in 18 of 20 samples collected from 20 samples in Malang City. Another study by Monikayani et al (2020) on Banjarmasin brand additional gallons of water and the most likely amount of additional water found that 5 out of 15 samples had the highest probability of having 2/100 ml coliform. Other researchers by Sudiana and Sudirgayasa (2020) regarding the Contamination Test of Coliform Bacteria and *E. coli* at the Refill Drinking Water Depot (DAMIU) in Tabanan District, Bali at 2 DAMIU with the 9 series tube method, both of which obtained MPN results of *E. coli* contamination of 93/100 ml.

Previous research by Mas and Pradina (2021) regarding Bacteriological Drinking Water Quality Tests in the Bukit Jimbaran Area at 3 DAMIU obtained positive results of *E. coli* indicating that the sample was not suitable for consumption. Research conducted by Atnafu et al (2021) regarding Microbial Community Structure and Diversity in Drinking Water Supply, Distribution System and Household Use Places in Addis Ababa City, Ethiopia on 38 samples collected from several different locations obtained dominant results from the genus *Pseudomonas*, *Legiolla*, *Klebsiella*, *Escherichia*, and *Actinobacteria*.

Based on previous research, researchers will conduct research to identify and calculate the number of pathogenic bacterial colonies using the Total Plate Count (TPC) method on refill drinking water in Cileungsi District, Bogor because until now there has been no Research on the content of pathogenic bacteria in refill drinking water which is sold along the road in Cileungsi District, Bogor.

METHOD

This research was conducted on February 14, 2022 to March 4, 2022 at the Bacteriology Laboratory of STIKes Mitra Keluarga. The sampling site for this research was conducted along the road in Cileungsi District, Bogor. The type of research used is descriptive research. The sampling method used is nonprobability with purposive sampling technique. In this study, bacteria were identified on endo agar media and counted the number of bacteria using the TPC method on refill drinking water.

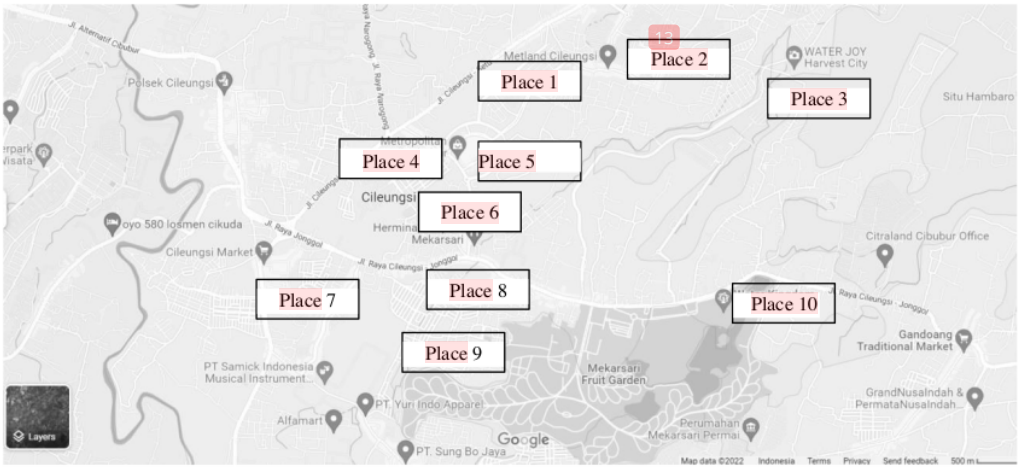

Figure 1. Locations along the road in Cileungsi District, Bogor indicate where to take refill drinking water Samples

Sterilize glass bottles to be used by wrapping them with clean paper tightly. The wrapped glass bottles were sterilized in an autoclave for 15 minutes at 121°C. The glass bottles then dry sterilized in an oven at 108°C for 2 hours (Hadijah, 2017). Eppendorf tubes were prepared as many as 3 tubes containing 900 μl NaCl in each tube. Then aseptically put the sample into the Eppendorf tube. The ratio of the sample weight to the volume of NaCl is 1:9. Then homogenize the sample and NaCl in the first dilution eppendorf tube. Pipette 100 μl from the first tube (10^{-1}) and transfer it to the second tube (10^{-2}) aseptically with the same ratio as before, which is 1:9. After that the sample was homogenized with NaCl in the second dilution eppendorf tube. The dilution was continued until the last dilution tube (10^{-3}) was followed by a similar method (Yusmaniar et al, 2017).

Planting on BHI-A Media, pipette 100 μl from each dilution tube (10^{0} to 10^{3}) in 2 petri dishes containing BHI-A and then flatten using drugalsky, the work is carried out in the BSC. Cover the edges of the petri dish tightly using plastic wrap in a circular motion. Then incubated in an incubator at 37°C for 1 ×24 hours. After calculating with the TPC formula, then take the colonies separated from the BHI-A media using a ose needle. Then do the streak plate technique with the quadrant stroke method. Then cover the edges of the petri dish using plastic wrap until there are no air voids (Aryal, 2022).

Colonies that were separated from the Endo Agar medium were taken using a ose needle for further biochemical tests. The colonies taken were colonies that grew in metallic green, pink, and white colors. Then do the streak plate technique with the continuous stroke method. Then cover it with a tube stopper and cover it with plastic wrap. For liquid biochemical test media, enter the durham tube aseptically and make sure that no air enters (Aryal, 2022).

Colonies were growing on Endo Agar media were taken and applied to slide preparations. Drops of crystal violet dye, let stand for 5 minutes, and rinse with running water and then dry. Add 12 drops of Iodine/Lugol (mordant) solution, let stand for 45-60 seconds, then rinse with alcohol until no longer purple, then rinse with water. Add the second dye, namely Safranin, let stand for 1 minute, rinsed with running water and dry. Add immersion oil and examine under a microscope with 40x magnification and 100x objective lens (Yusmaniar et al, 2017).

RESULTS

The characteristics of bacteria growing on BHI-A media were dominated by circular, white colonies with entire margins, colonies that were opaque and markedly elevated, but flat on the entire surface (raised). Classification of colonies from BHI-A media was carried out to make it easier for each colony to be isolated on Endo Agar media. This classification is based on almost the same characteristics in each DAM sample that grows and advances to ALT calculations.

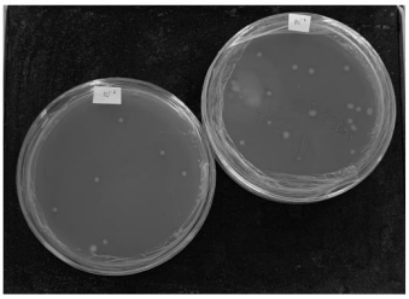

Figure 2. Colonies form of cultured bacteria from refill drinking water samples on BHI-A media

Table 1. Classification of Bacterial Colonies on BHI-A media

Kode Sampel	Karakteristik Koloni						
	Bentuk	Margin	Elevasi	Ukuran	Tekstur	Optik	Warna
A	*Circular*	*Entire*	*Flat*	*Large*	*Smooth*	*Opaque*	Putih
	Circular	*Entire*	*Flat*	*Large*	*Smooth*	*Opaque*	Putih
	Circular	*Entire*	*Flat*	*Moderate*	*Smooth*	*Opaque*	Putih
	Circular	*Entire*	*Flat*	*Moderate*	*Smooth*	*Opaque*	Putih
	Circular	*Entire*	*Flat*	*Small*	*Smooth*	*Opaque*	Putih
B	*Irregular*	*Curied*	*Flat*	*Large*	*Smooth*	*Opaque*	Putih
	Irregular	*Curied*	*Flat*	*Moderate*	*Smooth*	*Opaque*	Putih
	Irregular	*Curied*	*Flat*	*Moderate*	*Smooth*	*Opaque*	Putih
	Irregular	*Curied*	*Flat*	*Moderate*	*Smooth*	*Opaque*	Putih
	Irregular	*Curied*	*Flat*	*Small*	*Smooth*	*Opaque*	Putih
C	*Irregular*	*Undulate*	*Flat*	*Large*	*Smooth*	*Clear*	Putih
	Irregular	*Undulate*	*Flat*	*Large*	*Smooth*	*Clear*	Putih
	Irregular	*Undulate*	*Flat*	*Large*	*Smooth*	*Clear*	Putih
	Irregular	*Undulate*	*Flat*	*Moderate*	*Smooth*	*Clear*	Putih
	Irregular	*Undulate*	*Flat*	*Small*	*Smooth*	*Clear*	Putih
D	*Circular*	*Entire*	*Flat*	*Large*	*Smooth*	*Clear*	Putih
	Circular	*Entire*	*Flat*	*Large*	*Smooth*	*Clear*	Putih
	Circular	*Entire*	*Flat*	*Large*	*Smooth*	*Clear*	Putih
	Circular	*Entire*	*Flat*	*Moderate*	*Smooth*	*Clear*	Putih
	Circular	*Entire*	*Flat*	*Small*	*Smooth*	*Clear*	Putih

Classification of colonies from BHI-A media was carried out to make it easier for each colony to be isolated on Endo Agar media. This classification is based on almost the same characteristics in each DAM sample that grows and advances to ALT calculations.

Table 2. Calculation of the number of bacterial colonies using TPC method

Sampel Code	Bacteriological Quality of Drinking Water TPC (CFU/ml)	
	Result	Criteria
DAM 1	$5{,}5 \times 10^2$	TMS
DAM 2	$1{,}3 \times 10^2$	TMS
DAM 3	$0{,}5 \times 10^2$	MS
DAM 4	$1{,}2 \times 10^3$	TMS
DAM 5	$0{,}4 \times 10^2$	MS
DAM 6	$0{,}4 \times 10^2$	MS
DAM 7	$0{,}9 \times 10^3$	TMS
DAM 8	$1{,}05 \times 10^2$	TMS
DAM 9	$1{,}1 \times 10^3$	TMS
DAM 10	2×10^3	TMS

MS : Memenuhi Syarat
TMS : Tidak Memenuhi Syarat

Table 1 can be explained that MS (Qualify) and TMS (Not Qualify) of the 10 DAMIU in the Cileungsi sub-district, Bogor, all of which did not meet the bacteriological requirements in accordancewith Permenkes No. 492/MENKES/PER/IV/2010 based on the bacteriological requirements of drinking

water, which is not allowed to contain bacteria that exceed the predetermined threshold, which is 0/100 ml of water. According to SNI 6241:2015 regarding demineralized water obtained through the process of distillation, deionization, reverse osmosis, and purification by other processes equivalent to or without the addition of oxygen (O_2) or carbon dioxide (CO_2), the bacteriological requirement is 1.0×10^2, then DAMIUs that meet the standards are DAM 3 of 0.5×10^2 , DAM 5 of 0.4×10^2 , and DAM 6 of 0.4×10^2.

Table 3. One Way ANOVA test

ANOVA

ALT (CFU/ml)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	151363182000.000	9	16818131333.333	4.589	.013
Within Groups	36651340000.000	10	3665134000.000		
Total	188014522000.000	19			

The result of the One Way ANOVA statistical test obtained a p value of 0.01 (<0.05), which indicates that there is a significant difference in the amount of growth between samples of refill drinking water at depot.

Table 4. Bacterial colonies on Endo Agar media

Sample Code	Color	Result
A	Metallic green	*Escherichia coli*
B	Pink	Koliform
C	Pink	Koliform
D	Colorless	Non-koliform

Table 4 shows he coliform group is able to produce acid and gas from lactose fermentation. On Endo Agar, coliforms appear as red colonies with a metallic green luster. This unique color is caused by the production of aldehydes from lactose fermentation, the aldehydes can liberate fuchsin from the colorless Schiff reagent (fuchsin-sodium sulfite) making colonies appear red. In the case of *E. coli*, the reaction is so strong that fuchsin crystallizes giving colonies a metallic green sheen. Selective agents contained in the media, sodium desoxycholate and sodium lauryl sulfate help to inhibit non-colliform (Indrayati and Akma, 2018).

Table 5. Bacterial colonies on biochemical media

No	Media	Result			
		A	B	C	D
1	Simmon Citrate	−	+	+	+
2	Glucose	+	+	+	+
3	Lactose	+	+	+	+

Table 5 shows biochemical tests are used to determine whether refill drinking water samples are contaminated with pathogenic bacteria, especially *Escherichia coli* or not. In the biochemical reaction, there are 2 media used, namely Simmon Citrate Agar and Sugar-sugar media. In the media the sugars used are glucose and lactose.

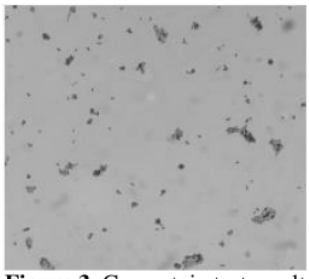

Figure 3. Gram stain test result

Colonies suspected of Escherichia coli were inoculated using ose with the characteristics of large colonies, convex elevation, smooth, pink in color with a metallic green luster (Indrayati and Akma, 2018).

DISCUSSION

Based on macroscopic observations from BHI-A media, it can be explained that of the 10 DAMIU in the Cileungsi sub-district, Bogor, all of which did not meet the bacteriological requirements in accordance with Permenkes No. 492/MENKES/PER/IV/2010 based on the bacteriological requirements of drinking water, which is not allowed to contain bacteria that exceed the predetermined threshold, which is 0/100 ml of water. According to SNI 6241:2015 concerning demineralized water obtained through the process of distillation, deionization, reverse osmosis, and purification by other processes equivalent to or without the addition of oxygen (O_2) or carbon dioxide (CO_2), the bacteriological requirement is 1.0×10^2 , then DAMIUs that meet the standards are DAM 3 of 0.5×10^2, DAM 5 of 0.4×10^2, and DAM 6 of 0.4×10^2.

Statistical data testing was carried out with the One Way Anova test. The results of the statistical test showed that there was a difference in the average number of TPC calculation results on the 10 DAMIU used as samples with a One Way ANOVA test significance of 0.01. If the probability (Sig < α) then Ho is rejected and Ha is accepted which indicates the difference between each depot. The result of the One Way ANOVA statistical test obtained a p value of 0.01 (<0.05), which indicates that there is a significant difference in the amount of growth between samples of refill drinking water at depot.

Characteristics of bacterial colonies based on morphology in BHI-A media in refill drinking water samples. Sample codes were ordered from DAM 1 to DAM 10. Bacterial colonies were then classified according to dominant characteristics. The characteristics of bacteria growing on BHI-A media were dominated by circular, white colonies with entire margins, colonies that were opaque and markedly elevated, but flat on the entire surface (raised). Classification of colonies from BHI-A media was carried out to make it easier for each colony to be isolated on Endo Agar media. This classification is based on almost the same characteristics in each DAM sample that grows and advances to the TPC calculation and then is coded for samples from A to D according to almost similar characteristics.

According to (Indrayati and Akma, 2018) the coliform group is able to produce acid and gas from lactose fermentation. On Endo Agar, coliforms appear as red colonies with a metallic green luster. This unique color is caused by the production of aldehydes from lactose fermentation, the aldehydes can liberate fuchsin from the colorless Schiff reagent (fuchsin-sodium sulfite) making colonies appear red. In the case of *E. coli*, the reaction is so strong that fuchsin crystallizes giving colonies a metallic green sheen. Selective agents contained in the media, sodium desoxycholate and sodium lauryl sulfate help inhibit non- coliform.

This study also identified using biochemical test are used to determine whether refill drinking water samples are contaminated with pathogenic bacteria, especially *Escherichia coli* or not. In the biochemical reaction, there are 2 media used, there are Simmon Citrate Agar and Sugars media. In the media the sugars used are glucose and lactose. Based on table 5, the results are (A) negative, (B) positive, (C) positive, and (D) positive. According to (Dewi et al, 2021) Simmon Citrate Agar is used to test the ability of organisms to utilize citrate as an energy source. Ammonium Dihydrogen Phosphate is the only nitrogen source. Dipotassium Phosphate acts as a buffer. Sodium Chloride maintains the osmotic balance of the medium. Sodium Citrate is the only carbon source in this medium. Magnesium Sulfate is a cofactor for various

metabolic reactions. Bacteria that can grow on this medium produce citrate permease enzymes that are able to convert citrate into pyruvate. Pyruvate can then enter the metabolic cycle of the organism for energy production. When bacteria metabolize citrate, the ammonium salt is broken down into ammonia, which increases alkalinity. The shift in pH changes the bromthymol blue indicator in the medium from green to blue above pH 7.6.

D-Glucose Broth media is a differential media. Tested an organism's ability to ferment glucose as well as its ability to convert the end product of glycolysis, pyruvic acid into a gaseous by-product. This is the test commonly used when identifying Gram-negative bacteria, all of which are glucose fermenters, but only a few that produce gas. If an organism is able to ferment glucose, an acidic byproduct is formed and the pH indicator changes color. The end product of glycolysis is pyruvate, an organism capable of converting pyruvate to formic acid and formic acid to H_2 (g) and CO_2 (g), through the action of the enzyme hydrogen lyase formate. This gas is trapped in the durham tube and appears as a bubble at the top of the tube. *Escherichia coli* can ferment glucose and produce gas.

Lactose broth gives excellent results for gas production at 45°C, which is characteristic of *Escherichia coli*. When preparing this medium, it is important to avoid overheating and distribute it into tubes prior to sterilization. Peptone and HM Peptone B in the medium are a source of nitrogen and carbon compounds, long chain amino acids, and other essential nutrients to organisms. Lactose is a fermentable carbohydrate for coliforms. Members of the coliform group are defined as gram-negative aerobic and facultativeanaerobes, which ferment lactose with gas formation.

Gram stain of *Escherichia coli* bacteria showed that bacteria with short trunks and red color after the staining process. This matter proved that Escherichia coli bacteria are Gram negative bacteria. According to Afrianti Rahayu and Muhammad Hidayat Gumilar (2017) this is due to the concentration of lipids and the thickness of the peptidoglycan layer on the bacterial cell wall. In Gram-negative cells, alcohol increases the porosity of the cell wall by dissolving the lipids of the outer layer. Thus, the first dye, i.e. Crystal Violet can be more easily removed from the peptidoglycan layer which is not tightly bound. Therefore, the leaching effect of alcohol results in the release of the unattached Crystal Violet complex, which renders the cells discolored or colorless. Only Gram-negative bacteria lose color, so their cells absorb Safranin as a red counter-dye.

CONCLUSION

Based on the results of the study, it can be concluded that the refill drinking water sold by the Refill Drinking Water Depot (DAMIU) in Cileungsi District is not in accordance with the Regulation of the Minister of Health Number 492/MENKES/PER/IV/2010 concerning Drinking Water Quality Requirements because it exceeds the threshold limit set by the Ministry of Health. has been set at 0/100ml. However, according to SNI 6241:2015 regarding the bacteriological requirements of demineralized water of 1.0×10^2, the DAMIU that meets the standards are DAM 3 of 0.5×10^2, DAM 5 of 0.4×10^2, and DAM 6 of 0.4×10^2. Pathogenic bacteria suspected of contaminating refilled drinking water are *Escherichia coli*, *Klebsiella sp*, and *Pseudomonas aeruginosa*. Allegedly due to contamination when refilling drinking water into gallons or containers used.

ACKNOWLEDGMENTS

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P/V You have used the passive voice in this sentence. Depending upon what you wish to emphasize in the sentence, you may want to revise it using the active voice.

PAGE 4

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Sp. This word is misspelled. Use a dictionary or spellchecker when you proofread your work.

PAGE 5

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S/V This subject and verb may not agree. Proofread the sentence to make sure the subject agrees with the verb.

Run-on This sentence may be a run-on sentence. Proofread it to see if it contains too many independent clauses or contains independent clauses that have been combined without conjunctions or punctuation. Look at the "Writer's Handbook" for advice about correcting run-on sentences.

Frag. This sentence may be a fragment or may have incorrect punctuation. Proofread the sentence to be sure that it has correct punctuation and that it has an independent clause with a complete subject and predicate.

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Missing "," You have a spelling or typing mistake that makes the sentence appear to have a comma error.

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Sentence Cap. Remember to capitalize the first word of each sentence.

Missing "?" Remember to use a question mark at the end of a question.

S/V This subject and verb may not agree. Proofread the sentence to make sure the subject agrees with the verb.