



Microbiological Safety of Bottled Water (10NS2)

DECEMBER 2011

SUMMARY

This survey investigated the microbiological safety and quality of bottled water, at manufacturing level and on retail sale in Ireland, by testing for the presence of *Escherichia coli*, enterococci, coliforms and *Pseudomonas aeruginosa*. Overall, 2.5% (19/748) of samples were categorised as unsatisfactory because one or more parameter was detected. This marks a significant improvement ($p < 0.05$) on the microbiological quality of bottled water surveyed by the Food Safety Authority of Ireland (FSAI) in 2007 (FSAI, 2008). Two percent (18/738) of samples (for which all four tests were carried out) were categorised as unsatisfactory in 2010, compared to seven percent (69/952) of samples in 2007.

Nevertheless, manufacturers of bottled water must continue to improve microbiological standards because four water samples were still considered unsafe for human consumption. They were contaminated with *E. coli* or enterococci, pointing to the possible presence of enteric pathogens. The affected batches were recalled by the manufacturer. A further four samples were positive for the opportunistic pathogen *P. aeruginosa*, which is considered to be a risk to profoundly immunocompromised patients in hospitals, but does not appear to be a risk for the general population (Scientific Committee of the FSAI, 2009). Intensive care units in Irish hospitals were notified and advised to remove any affected batches of bottled water if in use.

For three unsatisfactory samples (two positive for *E. coli* and one for *P. aeruginosa*), investigations showed that the affected batch had been distributed outside Ireland. The FSAI notified the European Commission who then issued alerts using its Rapid Alert System for Food and Feed (RASFF) to inform other Member States of the possible risk to human health from consuming these batches of water.

With regards to labelling, only 45% of natural mineral water or spring water samples complied with the legislative requirement to include both the name of the spring, and the place where the spring is exploited on the label.

To protect the safety and quality of bottled water, manufacturers should review their food safety management systems to ensure their product complies with the legislative microbiological criteria for bottled water. Manufacturers should also ensure their product complies with all legislative labelling requirements so that they provide consumers with sufficient information about the product. Importers of bottled water should ensure that the bottled water they place on the market in Ireland complies with the microbiological criteria and specific labelling requirements of European Communities (Natural Mineral Waters, Spring Waters and Other Waters in Bottles or Containers) Regulations, 2007 (S.I. No. 225 of 2007).

ACKNOWLEDGEMENTS

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ABBREVIATIONS

cfu/g	colony forming units per gram
FSAI	Food Safety Authority of Ireland
HPSC	Health Protection Surveillance Centre
I. S.	Irish Standard
NSAI	National Standards Authority of Ireland
RASFF	Rapid Alert System for Food and Feed
S. I.	Statutory Instrument
WHO	World Health Organization



INTRODUCTION

Although bacteria may be present in bottled waters at low concentrations they are usually harmless, and outbreaks of human illness associated with bottled water are infrequent compared to those linked to tap water (Scientific Committee of the FSAI, 2009). However, a 2007 study of the microbiological safety and quality of bottled water on sale in Ireland found that 7.2% of bottled water tested for *E. coli*, enterococci, coliforms and *P. aeruginosa* did not meet microbiological standards¹ or guidelines² (FSAI, 2008).

Pathogens which have caused outbreaks of human illness through the consumption of bottled water include *Vibrio cholerae*, *Salmonella* Kottbus, *Salmonella* Bareilly, *P. aeruginosa* and *Campylobacter jejuni* (Scientific Committee of the FSAI, 2009). Monitoring bottled water for specific pathogens is difficult because pathogens tend to be present intermittently and in very low numbers. Therefore, it is considered more effective to monitor the water for index and indicator microorganisms which may be present in the water in higher numbers. According to the World Health Organization, an 'index' is a microorganism or group of microorganisms which, if detected, points to the presence of pathogenic microorganisms, for example as an index of enteric pathogens. An 'indicator' is a microorganism, or group of microorganisms which, if detected, is a measure of the effectiveness of a process, for example a process indicator, or disinfection indicator (WHO, 2006).

If the index microorganisms *E. coli* and/or enterococci are detected, the water is considered unsafe for human consumption. This is because their presence points to the potential presence of enteric pathogens. If coliforms (indicator microorganisms) are detected this may indicate a problem with the quality of the water source or possible contamination during the bottling process. Water that tests positive for coliforms (but negative for *E. coli* and enterococci) is not considered unsafe for consumption because the presence of coliforms on its own is not a strong indication of the potential presence of enteric pathogens. However, the presence of coliforms does require careful investigation into the possible causes and requires an enhanced monitoring of finished product and source water for the presence of *E. coli* and enterococci.

Although the direct detection in bottled water of *P. aeruginosa* is not a significant health risk for the general population, it is an opportunistic pathogen associated with hospital-acquired infections in individuals that are profoundly immunocompromised. These include patients with profound neutropaenia, cystic fibrosis and severe burns (Scientific Committee of the FSAI, 2009; Kerr & Snelling, 2009). The most serious *P. aeruginosa* infections include bacteraemia, pneumonia, urosepsis, and wound infections (Kerr & Snelling, 2009). Suitable measures within hospitals, such as removing an affected batch of bottled water from use, can be adopted to reduce the risk of patients becoming infected (Eckmanns *et. al.* 2008; Scientific Committee of the FSAI, 2009).

Legally, there are three classes of bottled water: natural mineral water; spring water; and other water. European Communities (Natural Mineral Waters, Spring Waters and Other Waters in Bottles or Containers) Regulations,

¹ Microbiological standards are limits for bacteria in foods that are established by food legislation

² Microbiological guidelines are limits for bacteria in foods that are advisory but are not established by food legislation. They are limits that are achievable by adherence to good hygienic practice and good manufacturing practice



2007 (S.I. No. 225 of 2007) lays down the requirements for the marketing of these waters, including definitions, exploitation, treatment, microbiological criteria, chemical contaminants, sales descriptions and packaging.

Natural mineral waters and spring waters are taken from underground sources. Ground water differs from surface water in its microbiological, mineral and gaseous composition because the water has passed through subsoil and underlying rocks, and because the underground environment is devoid of light (Scientific Committee of the FSAI, 2009). S.I. No. 225 of 2007 does not allow disinfection treatment of natural mineral or spring waters, and lays down microbiological criteria which these waters must comply with at source and during marketing. Other waters are waters which are not marketed as 'natural mineral water' or 'spring water'. Manufacturers may use a disinfection treatment on 'other waters' such as ozonation, ultraviolet treatment, filtration or chlorination. There is no legislative restriction on manufacturers bottling tap water that has previously been chlorinated. The legislation also lays down microbiological criteria for other waters.

The Irish standard for packaged groundwater (NSAI, 2010) is designed to assist the industry produce water which meets the requirements of legislation. In addition, the FSAI has produced guidance for the enforcement of legislation applicable to natural mineral waters, spring waters and other bottled waters (FSAI, 2010).

OBJECTIVES

The purpose of this study was to examine bottled water for sale in Ireland for the presence of *E. coli*, enterococci, coliforms and *P. aeruginosa*.

METHOD

Sample Collection

Between September and December 2010, environmental health officers collected samples of natural mineral water, spring water and other water, supplied in bottles or containers, from bottled water manufacturers and retail outlets throughout the Republic of Ireland. Both effervescent and still water were included in this survey. Excluded from the survey were: flavoured water; unbottled water, e.g. tap water, water from dispensers at retail or catering level; and waters which are defined as medicinal products³. No more than one sample from each production batch was collected at each establishment. The minimum sample size was 1 L to provide enough water for four tests (*E. coli*, enterococci, coliforms and *P. aeruginosa*) to be carried out on each sample. If the sample was made up of more than one bottle, e.g. 4 x 250 ml bottles, the selected bottles came from the same production batch and were pooled in the laboratory before microbiological testing was carried out. All samples were transported to the laboratories for testing in a cool box. For each sample taken, environmental health officers were requested to complete a survey questionnaire (Appendix 1).

Sample Analysis and Classification of Results

Water samples were tested for: *E. coli*, enterococci, coliforms and *P. aeruginosa* (Table 1).

Table 1: Microbiological criteria applied in this survey

Test	Type of microorganism	Method ^a	Microbiological quality (cfu/g) ^b	
			Satisfactory	Unsatisfactory ^c
<i>E. coli</i>	Index	ISO 9308-1	0/250 ml	> 0/250 ml
Enterococci	Index	ISO 7899-2	0/250 ml	> 0/250 ml
Coliforms	Indicator	ISO 9308-1	0/250 ml	> 0/250 ml
<i>P. aeruginosa</i>	Pathogen	Pr EN ISO 12780	0/250 ml	> 0/250 ml

^a Analytical reference method specified in S.I. No. 225 of 2007

^b Criteria applied to natural mineral water and spring water at source and during their marketing. Criteria applied to other water at the point at which the water is put into the bottles or containers. However, other waters can be sampled at retail level for the purposes of check monitoring and analysed for these parameters (S.I. No. 225 of 2007)

^c Follow-up action was taken by environmental health officers if test result was unsatisfactory

Statistical Analysis

Chi square (χ^2) analysis or Fisher's Exact Test was performed using SPSS version 18.0, with significance defined at the alpha = 0.05 significance level. If the p-value is less than the alpha value of 0.05, there is a significant difference.

³ As defined in Directive 2001/83/EC (OJ L 311, p67, 28/11/2001) of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use (Directive 2001/83/EC 2001)

RESULTS AND DISCUSSION

Bottled water generally receives no further treatment by the consumer before consumption, so its microbiological safety and quality are of paramount importance. The microbiological quality and safety of bottled water is influenced by the microbiological status of the source water and the level of hygiene in the extraction and bottling process. S.I. No. 225 of 2007 lays down microbiological criteria for bottled waters with which manufacturers must comply.

Safety of Bottled Water and Action Taken on Unsatisfactory Samples

Nineteen (2.5%) of the 748 bottled water samples collected for this survey were categorised as unsatisfactory because one or more parameter⁴ was detected (Table 2). Investigations were carried out by environmental health officers and the food business operators as to the cause of the contamination. In all cases, the manufacturer was required to immediately investigate the cause of the unsatisfactory result and take any necessary corrective action to prevent reoccurrence. They were required to review their food safety management system and amend if necessary.

Four of these water samples were considered unsafe for human consumption because the index microorganisms *E. coli* or enterococci were detected, pointing to the possible presence of enteric pathogens. The manufacturers removed the affected batches from the food chain and notified trade customers and consumers⁵.

A further four samples tested positive for *P. aeruginosa*. A product recall was not required in this case as the presence of *P. aeruginosa* is not considered a significant health risk for the general population. However, it is considered a health risk to severely immunocompromised people, so the FSAI notified the HPSC who issued advice to intensive care units to remove the affected batches if in use.

Coliforms only were detected in eleven samples. The presence of coliforms indicates there may be a problem with the quality of the source water or possible contamination during the bottling process. Investigations into the possible cause were therefore carried out at the bottling plants by the manufacturers and environmental health officers. If the water was bottled in a country other than Ireland, the FSAI informed the regulatory authority in that country.

Finally, for three unsatisfactory samples (two positive for *E. coli* and one for *P. aeruginosa*), investigations showed that the affected batch had been distributed outside Ireland. The FSAI notified the European Commission's alert system (RASFF) which then issued alerts to inform other member states of the possible risk to human health from consuming these batches of water.

⁴ Note all four tests were not carried out on ten samples

⁵ In accordance with Article 19 of Commission Regulation (EC) No 178/2002

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Table 2: Details of samples with one or more unsatisfactory test results (n=19)

Sample number	Unsatisfactory for	Other test results	Water type	Country of origin	Action taken
1	<i>E. coli</i> (1 cfu/g) Coliforms (1 cfu/g)	Not performed ^a	Natural mineral water	UK	Product recall: Alert 2010.24. RASFF notification: 2010.1487 www.fsai.ie/ecoli_beechvaleywater.html
2	<i>E. coli</i> (1 cfu/g) Coliforms (1 cfu/g)	Satisfactory	Spring water	UK	Product recall: Alert: 2010.17. RASFF notification: 2010.1251 www.fsai.ie/news_centre/food_alerts/Springhill.html
3	Enterococci (71 cfu/g)	Satisfactory	Spring water	Ireland	Product recall: Alert 2010.18. RASFF not necessary as distributed in Ireland only www.fsai.ie/news_centre/food_alerts/braganspringwater.html
4	Enterococci (576 cfu/g)	Satisfactory	Other water ^b	Ireland	Product recall: Alert: 2010.20. RASFF not necessary as distributed in Ireland only www.fsai.ie/news_centre/food_alerts/celticpure.html
5	<i>P. aeruginosa</i> (7 cfu/g)	Satisfactory	Spring water	Ireland	Investigation carried out. HPSC notified intensive care units advising removal of affected batch if in use
6	<i>P. aeruginosa</i> (11 cfu/g)	Satisfactory	Natural mineral water	Ireland	Investigation carried out. HPSC notified intensive care units advising removal of affected batch if in use
7	<i>P. aeruginosa</i> (36 cfu/g)	Satisfactory	Natural mineral water	Ireland	Investigation carried out. HPSC notified intensive care units advising removal of affected batch if in use
8	<i>P. aeruginosa</i> (5 cfu/g)	Satisfactory	Natural mineral water	Lithuania	RASFF notification: 2010.1460. HPSC notified intensive care units advising removal of affected batch if in use
9	Coliforms (21 cfu/g)	Satisfactory	Other water	Ireland	Investigation carried out
10	Coliforms (3 cfu/g)	Satisfactory	Other water	Ireland	Investigation carried out
11	Coliforms (1 cfu/g)	Satisfactory	Natural mineral water	Ireland	Investigation carried out
12	Coliforms (1 cfu/g)	Satisfactory	Other water	Ireland	Investigation carried out
13	Coliforms (10 cfu/g)	Satisfactory	Other water	Ireland	Investigation carried out
14	Coliforms (1 cfu/g)	Satisfactory	Other water	Ireland	Investigation carried out
15	Coliforms (1 cfu/g)	Satisfactory	Spring water	Ireland	Investigation carried out
16	Coliforms (1 cfu/g)	Satisfactory	Other water	Ireland	Investigation carried out
17	Coliforms (15 cfu/g)	Satisfactory	Natural mineral water	UK	Referred to Food Standards Agency UK for investigation
18	Coliforms (8 cfu/g)	Satisfactory	Natural mineral water	UK	Referred to Food Standards Agency UK for investigation
19	Coliforms (3 cfu/g)	Satisfactory	Spring water	UK	Referred to Food Standards Agency UK for investigation

^a Neither the enterococci or *P. aeruginosa* test was performed on this sample; ^b This sample was taken at retail level and the microbiological criteria for other water applies at point at which the water is put into bottles or containers. However, the water was considered unsafe (as it contained enterococci) and so should not be placed on the market (Regulation 178/2002, Article 14)



Overall Microbiology Results

Of the 2,978 test results received^f, 0.7% (n=21) had unsatisfactory results: 0.3% *E. coli*, 0.3% enterococci, 1.7% coliforms and 0.5% *P. aeruginosa* (Figure 2). There was a significant improvement (p<0.05) in the microbiological results of bottled water tested in 2010 compared to 2007. Two percent (18/738) of samples (for which all four tests were carried out) were unsatisfactory in 2010 compared to 7% (69/952) in 2007. When the results of each of the four tests are analysed further, the improvement was driven by a significant improvement (p<0.05) in the results for coliforms (Table 3) indicating improvements to hygiene and/or source water quality.

Figure 2: Microbiological classification of tests carried out (n=2,978)

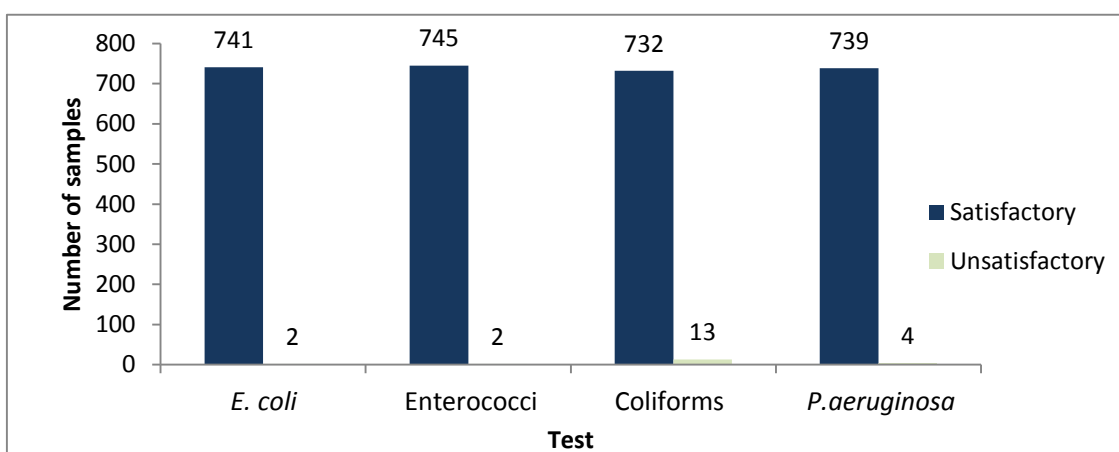


Table 3: Comparison of microbiology results of samples between 2007 and 2010

Test	% Unsatisfactory (number unsatisfactory/total tested)		Statistically significant difference? (p<0.05)
	2007	2010	
Overall (samples on which all four tests were carried out)	7.2 (69/952)	2.4 (18/738)	Yes
<i>E. coli</i>	1.0 (10/960)	0.3 (2/743)	No
Enterococci	0.2 (2/955)	0.3 (2/747)	No
Coliforms	6.3 (60/960)	1.7(13/745)	Yes
<i>P. aeruginosa</i>	0.8 (8/955)	0.5 (4/743)	No

^f 14 of the expected 2,992 tests (4 x 748) were not carried out: *E. coli* (5); enterococci (1); coliforms (3); and *P. aeruginosa* (5)



Questionnaire Data

Questionnaires were completed within the specified timeframe for 655 water samples; a response rate of 88%. In total, 553 samples were collected from retail establishments and 102 samples were taken directly from the bottling plant; 403 water samples were bottled in Ireland and 188 were bottled outside Ireland^g; 118 samples were effervescent and 537 were not. With regards to the sales description, 183 samples were labelled as natural mineral water, 140 samples were labelled as spring water and 291 samples had neither natural mineral water or spring water mentioned on the label and hence were designated as other water^h.

Overall, 2.3% (15/655) of samples for which a questionnaire was completed were unsatisfactory for one of more parameter. None of these samples were unsatisfactory for *E. coli*; however, nine samples were unsatisfactory for coliforms, four were unsatisfactory for *P. aeruginosa*, and two were unsatisfactory for enterococci. A breakdown of the unsatisfactory results by variable (sales description, effervescence, origin and sampling point) is provided in Appendix 2.

With regards to labelling, S.I. No. 225 of 2007 requires that the name of the spring and the place where the spring is exploited must be included on the label of water that is labelled as natural mineral water or spring water. Of the 323 water samples labelled as natural mineral water (n=183) or spring water (n=140), only 45% were labelled with both requirements. Water which is not labelled as natural mineral water or spring water can be considered other water, for which there are no specific labelling requirements under S.I. No. 225 of 2007.

CONCLUSIONS

There has been a significant improvement ($p < 0.05$) in the microbiological quality of bottled water on sale in Ireland since a survey carried out in 2007. Two percent (18/738) of samples tested for *E. coli*, enterococci, coliforms and *P. aeruginosa* were unsatisfactory in 2010 compared to 7% (69/952) in 2007. This was driven by a significant improvement ($p < 0.05$) in coliform test results, where 1.7% (13/745) were unsatisfactory in 2010 compared to 6.3% (60/960) in 2007. Although this indicates an improvement in hygiene and/or source water quality, manufacturers must continue to improve microbiological standards as four batches of water (two bottled in Ireland and two bottled in the UK) were required to be recalled from the market because they were considered unsafe for human consumption. A further four samples (three bottled in Ireland and one bottled in Lithuania) were positive for the opportunistic pathogen *P. aeruginosa*, which is considered a health risk for severely immunocompromised people.

With regards to labelling, 45% of water samples labelled as natural mineral water or spring water did not comply with the legislative requirement to include on the label, the name of the spring and the place where the spring is exploited.

^g Origin was not stated on the questionnaire for 64 samples

^h Conflicting answers were provided on the questionnaire for 41 samples so were regarded as spoiled answers

RECOMMENDATIONS

The recommendations of this survey are:

- 1) Irish bottled water manufacturers should review their food safety management systems to ensure they produce water which meets the microbiological criteria laid down in S.I. No. 225 of 2007
- 2) Irish bottled water manufacturers should ensure that products comply with the specific labelling requirements for bottled water laid down in S.I. No. 225 of 2007
- 3) Importers of bottled water should ensure that the bottled water they place on the market in Ireland complies with the microbiological criteria and specific labelling requirements of S.I. No. 225 of 2007.



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APPENDIX 1: SURVEY QUESTIONNAIRE

APPENDIX 3
FINAL Questionnaire 10NS2
Microbiological Safety of Bottled Water

Please note: 1) EHOs must complete this questionnaire for all samples, 2) all questions are mandatory & 3) all questionnaires must be returned to the FSAI by 11/02/2011 at the latest. Questionnaires must be returned via the webform on SafetyNet.

1. General Information

- * EHO Name: _____
- * EHO Sample Reference No. (i.e. EHO's own personal reference no. for the sample) _____
- * Lab: _____
- * Laboratory Reference No. (upon receipt of lab report) _____

2. Sample source (See section 3 of Protocol)

Bottled water manufacturer or Retail establishment

If retail establishment, please complete as appropriate, i.e.

CDC Supermarket Convenience store Pub Restaurant Hotel Other

Please specify _____

3. Sample Labelling

- i) Does the label carry the terminology: 'natural mineral water'? Yes or No
- ii) Does the label carry the terminology: 'spring water'? Yes or No

Only answer questions (iii) and (iv), if the answer to either question (i) or (ii) was **yes**

- iii) Does the label provide the name of the spring? Yes or No

If yes, please provide details: _____

- iv) Does the label provide the area of exploitation of the spring? Yes or No

If yes, please provide details: _____

4. Other sample information

Is the water effervescent (i.e. naturally carbonated, carbonated or fortified with gas): Yes or No

Is the water bottled in Ireland: Yes or No or Unknown

Sales description: _____

Trade/Brand name: _____

Bottlers name & address: _____

Batch Number: _____

Best before date: _____

5. Follow-up action (see section 11 of protocol):

(Follow-up action is only required for unsatisfactory results. Please tick as many boxes as necessary)

- None
- Product withdrawal
- Product recall
- Repeat sample Lab. ref. no. of repeat sample: _____
- Other Please provide details: _____



APPENDIX 2: MICROBIOLOGY RESULTS BY VARIABLE OF SAMPLES FOR WHICH A QUESTIONNAIRE WAS COMPLETED

Variable		Test	% unsatisfactory	Number unsatisfactory/ tested
Sales description	Natural mineral water	<i>E. coli</i>	0	0/181 ^a
		Enterococci	0	0/183
		Coliforms	1.6	3/183
		<i>P. aeruginosa</i>	1.7	3/181 ^a
	Spring water	<i>E. coli</i>	0	0/140
		Enterococci	0.7	1/140
		Coliforms	1.4	2/140
		<i>P. aeruginosa</i>	0.7	1/140
	Other water	<i>E. coli</i>	0	0/290 ^d
		Enterococci	0.3	1/291
		Coliforms	1.4	4/290 ^d
		<i>P. aeruginosa</i>	0	0/291
Effervescent	Yes	<i>E. coli</i>	0	0/118
		Enterococci	0	0/118
		Coliforms	0	0/118
		<i>P. aeruginosa</i>	0.9	1/116 ^a
	No	<i>E. coli</i>	0	0/534 ^c
		Enterococci	0.4	2/537
		Coliforms	1.7	9/536 ^d
		<i>P. aeruginosa</i>	0.6	3/537
Origin	Ireland	<i>E. coli</i>	0	0/403
		Enterococci	0.5	2/403
		Coliforms	1.5	6/403
		<i>P. aeruginosa</i>	0.7	3/403
	Outside Ireland	<i>E. coli</i>	0	0/185 ^c
		Enterococci	0	0/188
		Coliforms	1.1	2/187 ^d
		<i>P. aeruginosa</i>	0.5	1/186 ^a
Sampling point	Manufacturer	<i>E. coli</i>	0	0/102
		Enterococci	1.0	1/102
		Coliforms	2.0	2/102
		<i>P. aeruginosa</i>	2.9	3/102
	Retailer	<i>E. coli</i>	0	0/550 ^c
		Enterococci	0.2	1/553
		Coliforms	1.3	7/552 ^d
		<i>P. aeruginosa</i>	0.2	1/551 ^a
^a Result not provided for two samples ^b Result not provided for one sample ^c Result not provided for three samples				



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