

Incidence of antibiotic resistant pathogenic bacteria in vegetable items sold by local and super shops in Dhaka city

Ahmed Kabir, Ashish Kumar Das and Md. Shahidul Kabir[‡]

Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka 1217, Bangladesh

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The present study was carried out to investigate the microbiological quality and antibiotic resistance patterns of pathogenic bacteria isolated from vegetable samples. A total of 14 vegetable samples, 7 from local and 7 from super shops, were randomly collected from different locations of Dhaka city. Concentrations of total heterotrophic bacteria, total coliform, faecal coliform, *Pseudomonas* spp., *Listeria* spp. and *Staphylococcus aureus* were enumerated from each sample by serial dilution and spread plate technique. Presence of *Salmonella* spp., *Shigella* spp. and *Vibrio* spp. were determined by enrichment and selective plating methods. Antibiotic sensitivity patterns of the isolated bacteria were determined using Imipenem (10 µg), Ceftriaxone (30 µg), Sulphamethoxazole (25 µg), Ampicillin (10 µg), Gentamicin (10 µg), Aztreonam (30 µg), Cefuroxime (30 µg) and Oxacillin (5 µg) antibiotic discs. The local market vegetables showed higher proportions of *E. coli* (4/7, 57.14%) but the super shop vegetables showed higher proportions of *Pseudomonas* spp. (5/7, 71.42%) and *Listeria* spp. (5/7, 71.42%). Pathogenic bacteria isolated from the super shops showed increased resistance against (5/8, 62.5%) antibiotics tested against the pathogenic bacteria. Contamination of vegetables by a range of pathogenic bacteria in local and super market vegetables is a serious threat to public health if they are consumed raw or unprocessed. Higher antibiotic resistance in pathogens isolated from supermarket vegetables needs to be investigated in order to monitor and control spread of infections with drug resistant bacteria.

Key words: Vegetables; Contamination; Microbiological quality; Antibiotic resistance

Vegetables constitute a major part of our daily meal and meet the requirements of nutrients, vitamin and minerals. Although vegetables generally contain a load of 10^3 to 10^5 microorganisms/cm³ or 10^4 to 10^7 microorganisms/g, they may harbor a number of pathogenic microorganisms dispersed over the plants or micro colonies in the plant tissues (1). The predominant bacterial types found on vegetables are lactic acid bacteria, *Corynebacterium*, *Enterobacter*, *Proteus*, *Micrococcus*, *Enterococcus*, *Pseudomonas* and spore-formers. They may also possess different types of molds, such as *Alternaria*, *Fusarium*, and *Aspergillus* growing on their surface. Vegetables can be contaminated by enteric pathogens if animal or human wastes and polluted water are used for fertilization and irrigation (2, 3). Raw vegetables may be bruised during processing and distribution resulting in the release of plant nutrients which may serve as the essential organic and inorganic substrates for microorganisms (2, 4). Variety of microorganisms including pathogens can be

introduced from the surface of vegetables during processing of fresh salad vegetables. A large number of pathogenic microorganisms including *Salmonella*, *Escherichia coli* O157:H7, *Bacillus anthracis*, *Mycobacterium* spp., *Brucella* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, *Clostridium perfringens*, *Klebsiella* spp. and *Mycobacterium paratuberculosis* have been reported to be associated with contamination of vegetables. A number of bacterial diseases such as, diarrhoea, anthrax, salmonellosis, listeriosis, Crohn's disease and arthritis have been reported to be caused through consumption of contaminated vegetables by pathogenic microorganisms (5-8).

Vegetables can be contaminated from different environmental sources, such as soil, water, insects, air, birds, animal or equipment during cultivation and marketing. Pathogens present in contaminated foods may harbour virulence genes, toxins and enzymes, which aids in pathogenesis of infectious diseases (9, 10). In Bangladesh, food borne enteric disease is responsible for one third of childhood deaths each year from diarrhoeal diseases (11). Inception of supermarket for selling variety

[‡]Corresponding Author: Mailing address, Dr. Md. Shahidul Kabir, Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka 1217, Bangladesh; E-mail: mskabir@yahoo.com.

of food, vegetables and grocery items is new in the context of Bangladesh. There are few reports on different types of vegetables sold by the local and super markets. This study was therefore, conducted to determine the microbiological contamination of vegetable items sold by different outlets of local and super shops in Dhaka city.

MATERIALS AND METHODS

Sampling sites. Seven vegetable samples (Tomato, Cauliflower, Cabbage, Bean, Carrot, Radish, and Turnip) were aseptically collected from each of 7 local- and super-markets in Dhaka city. Samples were collected early in the morning in a sterile plastic bag and transported to the laboratory as soon as possible for further processing. This study was conducted between 1st February, 2014 and 15 April 2014.

TABLE 1. Description of the sites from where vegetable samples were collected

Sample ID	Sample type	Category of shop	Location of market
1-T-L	Tomato	L	Bonani bazar
2-T-S	Tomato	S	Swapno, Mirpur
3-CF-L	Cauliflower	L	Kawran bazar
4-CF-S	Cauliflower	S	Swapno, shawrapara
5-CB-L	Cabbage	L	Mirpur 6 bazar
6-CB-S	Cabbage	S	Meena bazar, Mohammedpur
7-B-L	Bean	L	Mohakhali bazar
8-B-S	Bean	S	Agora, Shanti nagar
9-CA-L	Carrot	L	Malibag bazar
10-CA-S	Carrot	S	Agora, Mog bazar
11-R-L	Radish	L	West Raza bazar
12-R-S	Radish	S	Agora, Dhanmondi
13-T-L	Turnip	L	Mirpur 11, Kacha bazar
14-T-S	Turnip	S	Meena bazar, Azimpur

L = Local market; S = Super market

Sample processing. All samples were processed following standard methods (12). 10 gm of vegetable samples were weighted and added into 90 ml of sterile normal saline in a blender and homogenized for 1-2 minutes before dilution and plating on both non-selective and selective media.

Inoculation and incubation of media. Samples were diluted up to 10^{-7} dilution following a 10-fold serial dilution technique and were spread on Nutrient Agar (NA) medium, *Pseudomonas* Agar (PA), Membrane Fecal Coliform (mFC) agar, Manitol Salt Agar (MSA) and *Listeria* Identification Agar (LIA) to determine total heterotrophic bacteria, *Pseudomonas* spp., total/faecal coliforms, *Staphylococcus aureus* and *Listeria* spp. respectively. All plates showing 30-300 colonies were used for quantitation of bacterial load as cfu/g (13). All media were purchased in dehydrated powder form from Himedia Laboratories Ltd., India.

Heterotrophic bacterial count. Samples were serially diluted up to 10^{-7} and 0.1 ml suspension of sample was inoculated on nutrient agar medium by using spread plate method for heterotrophic count of microbes. Then the plates were incubated at 37 °C for 24 hours. Any visible colonies were counted for determining the concentration of total heterotrophic bacteria.

Total coliform and faecal coliform count. 0.1 ml of the diluted samples were spread on mFC agar and incubated at 37 °C and 44 °C for 24 hours for total coliform and faecal coliforms, respectively. After incubation characteristic blue colonies were counted to determine the concentration of total and faecal coliform.

***Pseudomonas* count on *Pseudomonas* agar.** To enumerate the concentration of *Pseudomonas* spp., 0.1 ml suspension from each sample was spread on the surface of the *Pseudomonas* agar medium and was incubated at 37 °C for 24 hours. After incubation, the bluish green colonies were counted as total *Pseudomonas* spp.

Enumeration of load of *Staphylococcus aureus* and *Listeria* spp. To enumerate the concentration of *Staphylococcus aureus* and *Listeria* spp., 0.1 ml of suspension was spread onto MSA and LIA. Yellow colony with yellow halo

were determined as *Staphylococcus aureus* and grey-green colonies black centre and a black halo were considered positive for *Listeria* spp.

Enrichment and isolation of *Shigella* spp., *Salmonella* spp. and *Vibrio* spp. Selenite Cystine broth and Alkaline Peptone water were used for enrichment of *Salmonella/Shigella* spp. and *Vibrio* spp. Following enrichment 2-3 loop of enriched media were streaked on selective plates for isolation of pathogenic bacteria (12). Plates were incubated at 37 °C for 24 hours. Transparent or transparent with black centred colonies on SS agar were suspected colonies of *Shigella/Salmonella* spp. Sucrose fermenting or non-fermenting colonies were considered as suspected colonies of *Vibrio* spp.

Biochemical tests for identification of pathogenic bacteria. A series of biochemical tests such as, KIA, MIU, citrate, catalase, oxidase, MR and VP tests were done for identification of the suspected pathogenic bacteria (13, 14).

Antibiotic susceptibility test. Inoculum was prepared from a discrete colony in Trypticase Soya Broth (TSB). Bacterial cell suspension was prepared to match the concentration of inoculum to that of 0.5 McFarland standard. Bacterial suspension was inoculated on Mueller Hinton Agar (MHA) medium using sterile swab stick. Plates were dried for 15 minutes and commercially available antibiotic discs were applied on the plate surface. Imipenem (IPM) 10µg, Ceftriaxone (CRO) 30µg, Sulfamethoxazole (SXT) 25µg, Ampicillin (AMP) 10 µg, Gentamicin (CN) 10µg, Aztronam (ATM) 30 µg, Cefuroxime (CXM) 30µg and Oxacillin (OX) 5µg discs from Himedia Laboratories Ltd., India were used to determine the sensitivity of the isolated bacteria. The plate was inverted incubated aerobically at 37°C for 24 hours. Sensitivity of the isolated bacteria was determined according to the Clinical and Laboratory Standards Institute guideline (15).

RESULTS

Total bacterial load in all samples tested from local and super markets are shown in Table 2. Count of total heterotrophic bacterial (THB) ranged between 3.5×10^4 and 7.4×10^5 cfu/g in all samples. Total coliforms (TC) were absent in 3 (21.4%) samples out of 14 samples tested. None of the samples showed the presence of faecal coliforms (FC). *Staphylococcus aureus* could not be detected in 5 (35.7%) samples tested. 50% of the samples were contaminated with *Pseudomonas* spp. 9 (64.3%) samples were found to be contaminated with *Listeria* spp. Table 3 shows the presence of *Salmonella*, *Shigella* and *Vibrio* spp. following enrichment and selective culture as previously described. None of the samples showed the presence of *Salmonella* and *Shigella* spp. However, sample 3-CF-L and 10-CA-S showed the presence of *Vibrio* spp. Table 4 demonstrates the proportion of bacterial contamination as compared to the total number of isolates. Local market samples were mostly contaminated with *E. coli* (4/7, 57.1%) followed by *Listeria* spp. (3/7, 42.9%) and *Staphylococcus aureus* (3/7, 42.9%). Super market vegetables showed different pattern and were found to be contaminated with *Pseudomonas* spp. (5/7, 71.4%) and *Listeria* spp. (5/7, 71.4%).

Antibiotic sensitivity patterns of the pathogenic bacteria isolated in this study from both local and super market samples are shown in Table 5. Resistance against Imipenem (25%), Ceftriaxone (91.7%), Sulfamethoxazole (58.3%), Ampicillin (83.3%) and Cefuroxime (83.3%) were found to be higher in pathogens isolated from super shops than those of local market. Pathogenic bacteria isolated from local market vegetables showed higher level of resistance against other antibiotics such as, Gentamicin (38.5%), Aztronam (53.8%) and Oxacillin (33.3%) compared to those isolated from super shop vegetables.

TABLE 2. Bacterial load in vegetable sample collected from local and super shops.

Sample ID	Bacterial load (cfu/g)					
	THB	TC	FC	<i>Staphylococcus aureus</i>	<i>Pseudomonas</i> spp.	<i>Listeria</i> spp.
1-T-L	7.4×10^5	1.1×10^3	0	0	0	0
2-T-S	3.5×10^4	9.0×10^1	0	0	5.4×10^3	0
3-CF-L	4.8×10^5	1.2×10^3	0	2.8×10^3	5.4×10^4	0
4-CF-S	8.0×10^4	4.0×10^2	0	4.0×10^1	0	3.0×10^1
5-CB-L	3.2×10^5	5.0×10^2	0	8.0×10^2	0	1.6×10^3
6-CB-S	4.8×10^4	0	0	0	2.0×10^2	6.0×10^2
7-B-L	4.9×10^4	0	0	2.5×10^1	3.0×10^2	2.0×10^3
8-B-S	3.7×10^4	8.0×10^2	0	2.3×10^1	4.0×10^2	2.0×10^2
9-CA-L	5.1×10^4	1.6×10^2	0	3.0×10^2	4.1×10^2	1.4×10^2
10-CA-S	3.5×10^4	8.0×10^2	0	0	0	2.0×10^2
11-R-L	5.8×10^5	1.2×10^3	0	1.6×10^3	0	0
12-R-S	4.0×10^4	0	0	0	8.0×10^2	3.0×10^2
13-T-L	5.2×10^5	1.0×10^2	0	2.2×10^3	0	1.4×10^1
14-T-S	3.9×10^5	2.0×10^1	0	3.0×10^2	7.2×10^2	0

THB = Total heterotrophic bacteria; TC = Total coliform; FC = Faecal coliform

TABLE 3. Growth of *Salmonella*, *Shigella* and *Vibrio* spp. in vegetable samples

Sample ID	<i>Salmonella</i> and <i>Shigella</i> spp.	<i>Vibrio</i> spp.
1-T-L	-	-
2-T-S	-	-
3-CF-L	-	+
4-CF-S	-	-
5-CB-L	-	-
6-CB-S	-	-
7-B-L	-	-
8-B-S	-	-
9-CA-L	-	-
10-CA-S	-	+
11-R-L	-	-
12-R-S	-	-
13-T-L	-	-
14-T-S	-	-

- = Growth negative; + = Growth positive

TABLE 4. Proportions of pathogenic bacteria isolated from local and super shops

Organism	Local market, n (%)	Super market, n (%)
<i>Pseudomonas</i> spp.	2/7 (28.6)	5/7(71.4)
<i>Vibrio</i> spp.	1/7 (14.3)	1/7 (14.3)
<i>Listeria</i> spp.	3/7 (42.9)	5/7(71.4)
<i>Staphylococcus aureus</i>	3/7 (42.9)	1/7 (14.3)
<i>E. coli</i>	4/7 (57.1)	0/7 (0)
<i>Salmonella</i> and <i>Shigella</i> spp.	0/7 (0)	0/7 (0)

DISCUSSION

Outbreaks of human diseases associated with the consumption of raw fruits and vegetables have been increasing in the developing as well as developed countries of the world during the past decade. In Bangladesh, vegetables are common food regularly consumed with major meals. Salad vegetables are often taken as raw without cooking as a side menu in lunch or dinner. Consequently, consumption of contaminated vegetable may set outbreak of food borne diseases and

may put the overall public health at a serious risk. This could be linked to the fact that most of these vegetables are consumed without any thermal process or washing (16). The present study focuses on the determination of bacterial load and contamination of pathogenic bacteria in common vegetables sold by the two categories of shops in Dhaka city.

Microbial contamination of vegetables may result from various unsanitary practices during cultivation and marketing (17). These vegetables can become contaminated with pathogenic microorganisms during harvesting, through human handling and use of harvesting equipment, transport containers, wild and domestic animals. In another study it was reported that bacterial contamination of salad vegetables was linked to the fact that they are usually consumed raw without any heat treatment (18). Consequently, pathogens from human, animal and environmental sources could be detected in foods ready for consumption. Besides the spoilage bacteria, yeasts and mould on raw fruits and vegetable, pathogenic bacteria, parasites and viruses have also been reported (19).

Contamination of vegetables with coliform bacteria indicates the possible presence of other enteric pathogens which was very much apparent in this study. Faecal coliforms are thermo tolerant members of coliform group which indicate contamination of vegetables from faecal matter. Although *E. coli* is considered as the normal flora of the human intestines, some strains of *E. coli* have been linked to diarrhoea, gastro-enteritis and urinary tract infections (19). *Escherichia coli* and *Klebsiella* spp. are well known coliform bacteria that are frequently isolated from soil, water in the environment (20). *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. were also isolated from vegetables by other workers (17). In a previous study *E. coli* was found to be predominant in some salad vegetables which included coriander, carrot, radish, spinach, fenugreek and cucumber (18). This study showed that coliforms, *E. coli*, *S. aureus*, *Vibrio* spp. and

TABLE 5. Antibiotic sensitivity patterns of bacteria isolated from vegetables collected from local and super shops

Sample ID	Organism	IPM-10	CRO-30	SXT-25	AMP-10	CN-10	ATM-30	CXM-30	OX-5
3-CF-L	<i>Vibrio</i> spp.	I	R	R	S	S	R	I	ND
3-CF-L	<i>Pseudomonas</i> spp.	S	R	S	I	S	S	R	ND
5-CB-L	<i>S. aureus</i>	S	S	S	S	S	R	S	S
5-CB-L	<i>Listeria</i> spp.	R	S	S	R	S	S	I	ND
5-CB-L	<i>E. coli</i>	S	I	S	R	S	S	I	ND
7-B-L	<i>Pseudomonas</i> spp.	I	R	R	S	R	R	R	ND
7-B-L	<i>Listeria</i> spp.	S	I	S	R	R	R	R	ND
9-CA-L	<i>E. coli</i>	I	I	I	R	S	S	R	ND
9-CA-L	<i>Listeria</i> spp.	I	I	S	R	R	R	R	ND
11-R-L	<i>S. aureus</i>	S	R	S	R	S	R	I	S
11-R-L	<i>E. coli</i>	I	S	I	R	R	I	R	ND
13-T-L	<i>S. aureus</i>	S	R	S	S	S	R	R	R
13-T-L	<i>E. coli</i>	R	R	S	R	R	S	R	ND
Local market	Total resistance (%)	2/13 (15.4)	6/13 (46.2)	2/13 (15.4)	8/13 (61.5)	5/13 (38.5)	7/13 (53.8)	8/13 (61.5)	1/3 (33.3)
2-T-S	<i>Pseudomonas</i> spp.	S	R	I	R	S	I	R	ND
4-CF-S	<i>Listeria</i> spp.	S	R	R	R	S	R	R	ND
4-CF-S	<i>Staphylococcus aureus</i>	S	R	R	S	S	R	I	S
6-CB-S	<i>Pseudomonas</i> spp.	S	R	R	R	I	S	R	ND
6-CB-S	<i>Listeria</i> spp.	R	R	R	R	S	R	R	ND
8-B-S	<i>Pseudomonas</i> spp.	S	R	R	R	S	R	R	ND
8-B-S	<i>Listeria</i> spp.	R	I	S	R	S	S	R	ND
10-CA-S	<i>Vibrio</i> spp.	S	R	R	S	S	R	R	ND
10-CA-S	<i>Listeria</i> spp.	S	R	S	R	R	S	R	ND
12-R-S	<i>Pseudomonas</i> spp.	S	R	I	R	S	I	R	ND
12-R-S	<i>Listeria</i> spp.	R	R	S	R	S	S	I	ND
14-T-S	<i>Pseudomonas</i> spp.	S	R	R	R	S	R	R	ND
Super market	Total resistance (%)	3/12 (25.0)	11/12 (91.7)	7/12 (58.3)	10/12 (83.33)	1/12 (8.3)	6/12 (50.0)	10/12 (83.3)	0/1 (0.0)

IPM-10 = Imipenm (10 µg); CRO30 = Ceftriaxone (30 µg); SXT-25 = Sulfamethoxazole, 25 µg; AMP-10 = Ampicillin (10 µg); CN-10 = Gentamicin (10 µg); ATM-30 = Aztronam (30 µg); CXM-30 = Cefuroxime (30 µg); OX-5 = Oxacillin (5 µg); ND = Not Done; R = Resistant; S = Sensitive; I = Intermediate.

Pseudomonas spp. were common in vegetable samples which are concordant with previous study results (21).

Staphylococcal contaminations have been linked to carriage in nasal cavities of food handlers or by infected workers. Such contamination can be a serious threat to public health because of the ability of the pathogen to cause food-borne infections and food-borne intoxication (18). Salad vegetables such as carrots, cucumber, tomato and radishes have also been reported to be contaminated with Gram negative bacteria (1).

Fresh fruits and vegetables can be contaminated with waterborne pathogens such as, *Salmonella* spp., *Shigella* spp. and *Vibrio* spp. if they are treated with contaminated water. In this study, *Salmonella* spp. and *Shigella* spp. could not be isolated from the vegetable samples but two samples showed the contamination of *Vibrio* spp. According to the WHO (2002), contamination of food items with notable pathogens like *Salmonella* spp. and *Vibrio* spp. is now a major public health concern worldwide (22). *Salmonella* spp. has also been isolated from carrots, cucumber and lettuce collected from different markets and vendors in Nigeria and other parts of the world (20, 23).

The sanitary quality of the cultivation water,

harvesting, transportation, storage, and processing of the produce will directly influence the load and types of microorganisms in fresh vegetables (1). Bacterial contamination of fruits and vegetables has also been reported by other workers in a separate study conducted in India (18). Growth and survival of microorganisms in vegetables will depend on the availability of nutrients and inhibitors present on their intact their intact and bruised skins (24). Often microorganisms can gain entry through the stomata, lenticels, broken trichomes and stem scars and may survive (25).

Prevalence of pathogenic bacteria in supermarket samples slightly varied from that of local market. The proportions of *Pseudomonas* spp. (71.4%) and *Listeria* spp. (71.4%) were relatively higher in the super shop samples compared to those of local shops. On the contrary, proportions of *Staphylococcus aureus* (14.3%) and *E. coli* (0%) were found to be lower in supermarket samples. These results indicate the better management of vegetables in supermarkets in relatively better handling and preservation of vegetables in chillers possibly helped to reduce the contamination of *S. aureus* and *E. coli*. However, survival ability of *Listeria* spp. and *Pseudomonas* spp. at refrigeration temperature could be

responsible for the increased concentration of these pathogens. Sometimes resistant bacteria such as, *Pseudomonas* spp. can survive in disinfectant solutions and build up their concentration in those solutions. Repeated use of these contaminated sanitizers might have helped increase the proportion of resistant bacteria in supermarket samples.

Overall, this study revealed the presence of a high load of microorganisms in the commonly consumed vegetables items in both local market and super shop among in Dhaka city. All samples from both the categories showed THB which ranged between 3.5×10^4 and 7.4×10^5 cfu/g. Although total coliforms could be detected in 11/14 (78.6%) samples, none of the samples showed the presence of faecal coliforms in this study. This result indicates that the contaminating bacteria were introduced to the vegetable samples from an environmental source and not from any faecal source. Future study could be done for tracking the source of contamination present in the environment. Pathogenic bacteria might be introduced from the cultivation land, organic fertilizers, irrigating water, packaging materials and transport vehicles.

It is important to determine the antibiotic resistance patterns of the pathogenic bacteria for appropriate treatment of infectious disease. Increase in the resistance pattern will complicate the treatment options and increase the cost of treatment and patient management. In this study, pathogenic bacteria isolated from the supermarket showed higher level of resistance against most of the common antibiotics tested compared to those of local market. It will be necessary to investigate the causes of this increased resistance in pathogens isolated from supermarkets. Frequent application of disinfectants and related chemicals used for sanitation and cleaning may be related to the increase in the antibiotic resistance amongst pathogenic bacteria. Increased antibiotic resistance amongst the pathogens isolated from super market vegetables is a great concern for the consumers and the society. Contaminating pathogens are capable of causing various types of enteric diseases as well as serious intoxications in human health. Further studies need to be done for detection of the virulent genes in the pathogenic bacteria for predicting and determining the cause of outbreak of diseases. It is also necessary to create awareness among the producers, processors, handlers and consumers of raw vegetables through campaign and related social activities. Such activities will help reduce the bacterial contamination and risk of illness associated with the consumption of contaminated vegetables. Improvement in the health and hygiene condition during vegetable production, handling, storage and distribution will be necessary to control foodborne diseases due to the consumption of

contaminated vegetables.

CONCLUSION

In this study microbial load in vegetable samples collected from the local and super shops demonstrated high load of THB, TC, *S. aureus*, *Pseudomonas* spp. and *Listeria* spp. Only 2 out of 14 samples showed the presence of *Vibrio* spp. Antibiotic resistance pattern was higher amongst the pathogens isolated from super shop samples. This is a matter of concern for the new types of shops under operation in Dhaka, Bangladesh. Necessary steps should be taken to reduce the level of contamination in vegetables sold either in local or super shops. It will be also necessary to create awareness among the vegetable producer, businessman and general public to significantly reduce the propagation and spread of antibiotic resistance bacteria. A larger scale study will be necessary to determine the sources of pathogenic bacteria and antibiotic resistant trait and to suggest necessary steps to be taken for appropriate intervention.

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