

Review study on external-hospital bacteria as a source of infection and antimicrobial resistance in Lebanon

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Abstract

Most studies done on bacteria in Lebanon are on hospital isolates. However, some of the sources of hospital isolates might be from contaminated foods and water that are imported into hospitals. Studies done in the Department of Experimental Pathology, Immunology and Microbiology at the American University of Beirut on bacteria isolated from seafood, fruits, vegetables and poultry are reviewed, and attempts made to isolate *V. cholerae* from various water sources is reported. The use of antibacterial agents as food additives for poultry as a contributing factor for the increase in resistant isolates is demonstrated. Methods to decontaminate foods prior to getting into the kitchens of hospitals and homes are recommended.

Introduction

Most studies dealing with bacteria as infectious agents, and antibacterial resistance in Lebanon were done on hospital isolates. However, the source and resistance of some of these microorganisms in hospitals could be external. According to a Center for Disease Control and Prevention (CDC) report 48 million people get sick, 128000 are hospitalized, and 3000 die of foodborne diseases annually [1]. Contaminated foods could be fruits, vegetables and poultry all of which have direct contact with soil. Moreover, contaminated seafood and drinking water could be a source of infection. More often than not, contaminants may be resistant to antibacterial agents.

In as much as resistant genes are concerned a recent report indicated that they all originated from the soil microbiota. Forsberg et. al. [2] reported that resistant genes originated from the soil microbiota and proposed that soil microbiota is the reservoir of resistant genes available for exchange with clinical pathogens. They provided evidence for exchange of antibiotic resistant genes between environmental bacteria

and clinical specimens. Multi drug-resistant soil bacteria (resistant to beta-lactams, aminoglycosides, sulfonamides, and tetracyclines) had perfect nucleotide identity to genes from human pathogens.

A source of resistant bacteria comes from livestock. Gyles [3] reported that increased exposure of intestinal bacterial flora to antibacterial agents used as a food additive is one of the causes for the emergence of resistant strains. As a result of a number of reports concurring with that of Gyles, the European Union decided to abolish the use of antibacterial agents as food additives for livestock and poultry. As a consequence of this decision it was later reported that there was a decrease in antibacterial resistance to zoonotic bacteria [4]. Unfortunately, in some countries including Lebanon, farmers still use antibacterial agents as food additives for their livestock and poultry.

This report will deal with a review of results obtained in the author's laboratory pertaining to food and water microbiology.

Vibrio species isolated from seafood

Species belonging to the *Vibrio* genus are known to cause disease. *V. parahemolyticus* when ingested causes an acute gastroenteritis. It is a seafood-borne infection. Individuals get infected by eating raw or undercooked shell fish [5]. *V. alginolyticus* is a member of the marine flora. It causes wound infections, otitis media and otitis externa. Individuals get infected when swimming in the sea or in the case of wound infections when one cuts him/herself while handling shell fish [6]. Two serogroups, O1 and O139 of *Vibrio cholerae* when ingested with drinking water cause a profuse watery diarrhea [7].

Referring to **Table 1**, out of 170 seafood specimens investigated at our laboratories; *V. parahemolyticus* was isolated from 2 fish and one crab specimens and *V. alginolyticus* was isolated from 7 fish, 3 shrimp, 2 sea urchins and 1 crab specimen [8].

Attempts to isolate *Vibrio cholerae* from various water sources in Lebanon

Water and plankton samples from 9 rivers, 4 springs and 1 lake were investigated (**Table 2**). Forty five specimens obtained from different water sites contained bacteria that formed yellow colonies on Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS agar), a characteristic of *Vibrio sp.* Gram staining indicated that the bacteria were mainly Gram negative rods or coccobacilli, another characteristic of *Vibrio sp.* DNA was extracted from the 45 yellow producing-colony isolates and the polymerase chain reaction (PCR) was performed using primers that amplify the *OmpW* gene that codes for the outer membrane protein of *V. cholerae* and is species specific. A 200 bp rather than a 304 bp band was obtained from amplified DNA obtained from 13 out of the 45 isolates. While the 32 remaining isolates are probably other *Vibrio* species that also form yellow colonies on TCBS agar. Another test that is reported to be *V. cholerae*-specific is the string test. One of the 13 200bp-positive isolates was string test-positive.

The 13 isolates that were 200bp-positive, in particular one of them that were string test-positive might be a mutant of *V. cholerae*. Further molecular studies and pathogenic studies in an animal model must be done to determine if these isolates or some of them are *V. cholerae*.

Bacterial flora of fruits and vegetables in Lebanon

Fruits and vegetables including strawberries, green almond, green gages, cherries, plums, peaches, pears, apples, radish, lettuce, mint, carrots and parsley were tested for their bacterial content [9]. Bacteria spp. belonging to 9 different genera was identified (**Table 3**). As expected vegetables that are exposed to soil had higher bacterial counts than the fruits that grow on trees. This observation is of significance in relation to Forsberg et. al. [2] report indicating that resistant genes originate from soil microbes. The number of organisms isolated prior to and after washing of the specimen tested per gram was determined. Washing reduced the number of organisms but did not eliminate them [9].

Out of the 11 serotypable *E. coli* isolates recovered, 4 had serotypes (O26, O44, O86 and O214). These serotypes have been reported to be enteropathogenic [10]. Recently, Burjaq and Shehabi reported on *E. coli* isolated from green vegetables in Jordan, some of which are known to be pathogenic [11].

Poultry samples

Bacterial species isolated from poultry

Six layer farms were studied. In 4 of the 6 farms, chickens received antibacterial agents as a food additive. One hundred and eleven cloacae swabs were collected and cultured on MacConkey agar and the colonies obtained were identified using the API 20 kit. We searched for Gram negative rods excluding *Salmonella* and *Shigella spp.* A number of species were identified (**Table 4**).

Table 1. *Vibrio parahemolyticus* and *Vibrio alginolyticus* isolated from seafood

Number of							
<i>Vibrio parahemolyticus</i> isolates from				<i>Vibrio alginolyticus</i> isolates from			
Fish	Crab	Shrimps	Sea Urchins	Fish	Crab	Shrimps	Sea Urchins
2	1	0	0	7	0	3	2
One hundred and seventy seafood specimens were tested							

Table 2. Water sources, growth of yellow colonies on TCBS agar, Gram stain of isolates, sucrose fermentation and amplification of the ompW gene.

Source of water sample	Number of yellow colonies on TCBS	Gram stain	Sucrose test	PCR of ompW gene
Tannourin spring	1 colony	Gram negative bacilli	positive	no bands
Safa spring	1 colony	Gram negative bacilli	positive	no bands
Barouk spring	12 morphologically different colonies	Gram negative bacilli	positive	2 bands for 2 different colonies
Labweh spring	2 morphologically different colonies	Gram negative bacilli	positive	no bands
karoun lake	4 morphologically different colonies	Gram negative bacilli	positive	no bands
Beirut river	4 morphologically different colonies	Gram negative bacilli	Positive	1 band
Al- kaleb river	7 morphologically different colonies	Gram negative bacilli	Positive	6 bands for 6 different colonies
Aowali river	3 morphologically different colonies	Gram negative bacilli	Positive	No bands
Ibrahim river	2 morphologically different colonies	Gram negative bacilli and coccobacilli	Positive	No band
Qassmeih river	2 morphologically different colonies	Gram negative bacilli and coccobacilli	Positive	1 band
Damour river	2 morphologically different colonies	Gram negative bacilli	Positive	2 bands for 2 different colonies
Assi river	3 morphologically different colonies	Gram negative bacilli	Positive	1 band
Litani river	1 colony	Gram negative bacilli	Positive	No band
Hasbani river	1 colony	Gram negative bacilli	Positive	No band

Relationship between poultry antimicrobial agent food additives and antimicrobial resistance

The predominant isolates from farms that used antibacterial agents as a food additive and farms that did not were *E. coli* (82 isolates from 111 specimens). They were used for antimicrobial susceptibility testing and it was determined that there was an association between the use of tetracycline or gentamicin as a food additive and the number of *E. coli* isolates resistant to these antibacterial agents (Table 5) [12].

Detection of virulence genes carried by resistant *E. coli* isolates

It was determined that among the 99 *E. coli*-resistant isolates 9 possessed enterotoxigenic (ETEC) and 4 possessed enteropathogenic (EPEC) genes [13].

Isolates and nosocomial infections

At least 4 bacterial species that have been isolated, in our studies (9, 12), from vegetables, fruits and poultry have been reported by Weinstein et. al. [14] to be causes of nosocomial infections. Antimicrobial susceptibility testing was not performed on isolates obtained from fruits and vegetables (Table 6). However, as mentioned the number of resistant and susceptible poultry *E. coli* isolates to tetracycline and gentamicin are given in Table 5.

PulseNet Middle East

PulseNet Middle East established in Lebanon since 2006 deals with investigating human foodborne diseases by confirming and tracking the source of infection. Outbreaks are initially identified by the epidemiology surveillance unit of the Ministry of Public Health and bacterial agents are subjected to phenotypic testing and genotyping using Pulsed Field Gel

Table 3. Common bacteria spp. isolated from fruits and vegetables samples.

Bacteria
Enterobacter spp
Citrobacter spp
Klebsella spp
Proteus spp
Pseudomonas spp
Providencia spp
Escherichia coli
Staphylococcus spp
Salmonella paratyphi B

Table 4. Bacteria isolated from cloacae of chickens.

Bacteria	Number of isolates
<i>Escherichia coli</i>	122
<i>Klebsella pneumonia</i>	9
<i>Enterobacter cloacae</i>	7
<i>Proteus vulgaris</i>	8
<i>Proteus mirabilis</i>	27
<i>Pseudomonas aeruginosa</i>	1
<i>Pseudomonas putida</i>	6
<i>H. olie</i>	1
<i>Enterobacter sakozaki</i> (<i>Cronobacter sakozaki</i>)	1
<i>Citrobacter freundii</i>	2
<i>Providencia rettgeri</i>	1

Table 5. Number of *E. coli* isolates resistant and susceptible to tetracycline and gentamicin when used as antibiotic growth promoters.

	No.(%)of isolates resistant to AGP	No.(%)of isolates susceptible to AGP	Total no. (%)of <i>E.coli</i> isolates
Tetracycline as AGP	49(59.8)	1 (1.2)	82(100)
Tetracycline not an AGP	10 (12.2)	22 (26.8)	
Gentamicin as AGP	20 (24.4)	13 (15.9)	82(100)
Gentamicin not an AGP	2(2.4)	47 (57.3)	

AGP: Antibiotic growth promoter.

Relative risk for Tetracycline= 19.1; 95% Confidence interval= 2.8 to 130.3.

Relative risk for Gentamicin= 4.2; 95% Confidence interval= 2.5 to 6.9.

Table 6. Gram negative isolates reported to cause nosocomial infections.

Isolate	Source
<i>Escherichia.coli</i>	vegetables, fruits, poultry
<i>Klebsiella pneumoniae</i>	vegetables, fruits, poultry
<i>Enterobacter species</i>	vegetables, fruits, poultry
<i>Pseudomonas aeruginosa</i>	vegetables, fruits, poultry

Type of hospital acquired infections: pneumonia, bacteremia, urinary tract infections.

Electrophoresis at the American University of Beirut. Generated data so far demonstrated the prevalence of particular strains belonging to various *Salmonella* species.

A number of Salmonellosis sporadic cases and outbreaks that occurred in 2011-12 in Lebanon were confirmed. Several outbreaks caused by *Salmonella* species were detected throughout Lebanon districts. *Salmonella enteritidis* was the most common serotype identified in these outbreaks followed by *Salmonella typhimurium*. A single genotypic pattern by Pulsed Field Gel Electrophoresis (PFGE) was detected per species per outbreak, denoting implication of a single strain in each outbreak. As for antimicrobial resistance, an outbreak in the Nabatiyeh district showed *Salmonella typhimurium* resistant to Ampicillin while susceptible to Ciprofloxacin, Cef-tazidime, and Trimethoprim Sulfamethoxazole. The rest of the outbreaks in various parts of Lebanon showed no antimicrobial resistance. More recently *E. coli* and *Shigella* species have also been encountered [15].

Concluding and recommendation remarks

Our studies indicated that there were a number of different potential gastrointestinal pathogens found in seafood, vegetables, fruits, chickens and possibly water. Lebanese populations are good consumers of all of these foods.

Our report about 29 years ago on the bacterial flora of fruits and vegetables, and more recently on poultry can be related to the recent report by Forsberg et. al. [2] who showed that resistant genes originated from soil microbiota. Therefore, bacteria isolated from specimens that have direct contact with soil might be carrying resistant genes.

Foods carrying resistant potential human pathogens are destined to kitchens at homes and hospitals, and especially in hospitals they might become a source of nosocomial infections.

To reduce the incidence on the development of resistant bacteria, the European Union implemented a law abolishing the use of antibacterial agents as food additives for livestock. The Lebanese authorities should implement a similar law. Moreover, food decontamination methods and proper personal handling practice should be introduced and controlled in each hospital. It is important that each country and organization to implement the suitable methods of food decontamination including treatment with chemicals such as lactic acid or citric acid, or exposure to ionizing radiation, pulse light, ozone or energetic reactive gases [16-20].

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