Possibility of Symbiosis between Some Gram-negative Bacteria and *Legionella pneumophila*

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Abstract: One of the biotic factors that affect *Legionella* survival and multiplication is the presence of other organisms. Most documents mentioned to the intracellular proliferation of *Legionella* in amoebae and ciliates. It is important to define the relationship that may exist between *Legionella* and other bacteria and the possibility of growth extracellulary in unsterile tap water. The basic experiments involved a comparison for the changes in numbers of *Legionella pneumophila* that was inoculated alone in sterile dechlorinated tap water with that resulted from culturing the same strain in the presence of by-products of culturing four different gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 15142; *Proteus mirabilis* ATCC 14153; *Escherichia coli* ATCC 14229 and *Acinetobacter baumannii* ATCC 19606) separately in sterile tap water. The results revealed somewhat variable stimulation effect for bacteria by-products on *Legionella pneumophila*. The qualitative as well as quantitative variations in the bacterial by-products as a function of variations in strain used and the period allowed to produce the by-products are the variables that affect the results. The first day by-products supporting ability can be arranged in the following descending order: *Prot. mirabilis – Ps. aeruginosa – A. baumannii*. *E. coli* by-product has no supporting activity. From the second day till 25th day the descending order appeared as: *Ps. aeruginosa – E. coli – A. baumannii – Prot. mirabilis*.

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INTRODUCTION

Water and moist environments may be the natural habitat for *Legionella pneumophila*, the causative agent of Legionnaires' disease. The principal route of these bacteria transmission is thought to be by inhalation of contaminated aerosols [1, 2]. One of the important factors that should be considered for studying the spread of pathogens through water is the survival of the causative agent which in turn depends on many abiotic factors such as pH, temperature, and nutrients availability [3]. Some pathogens known to survive in low-nutrient waters include *Pseudomonas cepacia* [4], *Pseudomonas aeruginosa* [5], *Legionella pneumophila* [6], *Salmonella typhimurium, Yersinia enterocolitica, Shigella* sp., and enteropathogenic *Escherichia coli* [7, 8]. A study on suspension of *Legionella pneumophila* in sterile distilled and tap water showed longterm survival but no evidence of multiplication [9]. On the other hand, it was demonstrated that naturally occurring *L. pneumophila* multiplied in tap water at 32^{o}, 37^{o} and 42^{o}C [10, 11]. Furthermore, it was reported that *Legionellae* is unable to proliferate in an aquatic environment without their hosts or perhaps complex diverse biofilms. They require preformed amino acids as carbon and energy sources [12].

Concerning the role of biotic factors that may support the growth of *L. pneumophila* in water, Tison *et al.* [13] concluded that the high rate of multiplication of *L. pneumophila* was dependent on active photosynthesis of cyanobacteria. Accordingly, under darkness conditions, which occur in plumbing systems, the cyanobacteria may not be able to support the growth of *L. pneumopila*. Factors other than cyanobacteria photosynthesis may be involved in providing the nutrients necessary for the growth of *Legionellae* in tap water as well as in plumbing systems. Several studies have shown that aquatic protozoa, especially amoebae, can provide the intracellular environment required for the replication and persistence of *Legionellae* [14-18]. It might be that the biofilm formed on pipe walls support the survival and growth of *Legionellae* outside a host cell [19]. *Legionella* resistant to high temperature and entrapment in the biofilm give it an advantage to survive in the hot water pipe lines as well as water storage tanks at homes and hospitals. Static water in building networks is often at warm-water temperatures that stimulate growth in the accumulated sediments [20]. The study of Murgan *et al.* [21] using a biofilm reactor suggested that *L. pneumophila* may persist in the absence of amoebae, but in a model potable water system, the amoebae were required for multiplication of the bacteria.

Only a few studies have attempted to characterize the interactions between water bacteria and *Legionella* in such diverse habitats as free water and biofilms [17].

The satellite growth study demonstrated that ***Flavobacterium breve*** can support growth of a subculture of *L. pneumophila* on an L-cysteine deficient medium [22]. In another study by the same authors [23], suspensions of different density of isolates mixture of non-*Legionellaceae* bacteria appear to enhance the survival or cryptic growth of agar grown *L. pneumophila*. High density (10^8 CFU/ml) of non-*Legionellaceae* caused a decline in *L. pneumophila* numbers within the first week of incubation. Naturally occurring *L. pneumophila* was multiplied in the presence of associated bacteria.

Such information may aid in the design of control measures aimed at preventing or elimination *Legionella* multiplication and spread of Legionnaires' disease and add basic knowledge concerning the ecology of *Legionella*. In the present study, we examined the role of the by-products resulted from sterile water cultured with four gram-negative bacterial species in supporting the multiplication of agar grown strain of *Legionella pneumophila* type 1 (ATTC 33152).

MATERIALS AND METHODS

Four ml of 24 hr broth cultures of *Escherichia coli* (ATCC 14229); *Proteus mirabilis* (ATCC 14153); *Acinetobacter baumanii* (ATCC 19606) and *Pseudomonas aeruginosa* (ATCC 15142) were centrifuged at 4000 r.p.m for 10 min. The sediment of bacteria was then re-suspended in the phosphate buffer and inoculated separately in 3 liter sterile flasks contained 2 liter of autoclaved tap water and kept on a shaker at room temperature (18 – 20^oC). Interevally, 50 ml sample of each flask were filtered through 0.2 µm pore size membrane filter (Sartoriüs A.G.W 3400, Göttingen-Germany). The resulted sterile filtrate resulted from every strain was transferred to a sterile 100 ml screw cap bottles. Each bottle was inoculated with 0.5 ml of *Legionella pneumophila* type 1 (ATCC 33152) suspension which resulted from picking five isolated colonies from cultured Buffered Charcoal Yeast Extract (BCYE) agar, Oxoid plates, suspended in 5 ml of phosphate buffer and vortex mixed. The same inoculum's of *L. pneumophila* was held in 50 ml sterile tap water as a control. All the inoculated bottles were incubated on a shaker at room temperature. The changes in *L. pneumophila* counts were checked by periodically transfer a 1.0 ml from the inoculated bottles to 9.0 ml phosphate buffer, serially diluted and from each of three dilutions, 2.0 ml was subcultured on BCYE agar plates, incubated at 37^o C for 24 -72 hr and *Legionella* colonies on the un-crowded plates were counted as mean figures and expressed as a colony forming units (CFU) / ml. At the same time, and as controls, 0.2 ml of each of the four bacterial species stock water culture was subcultured on pre-prepared McConkey agar (Oxoid) plates, and counted after incubation at 37^o C for 24 hr.

RESULTS

The possibility of supporting the multiplication of agar grown *Legionella pneumophila* strain was investigated in the presence of bacteria by-products resulted from inoculation of agar grown four laboratory stock cultures of gram-negative bacteria separately in sterile tap water. The changes in *L. pneumophila* counts resulted from inoculation in bacteria by-products were determined. Along the study period, there are some evidence supporting the phenomenon of *L. pneumophila* multiplication. The ability of bacteria by-products to support *Legionella* growth was varied and depend on bacterial species, and the age of by-product used. The first day filtrates resulted from the four tested bacterial species, except *E, coli*, could support *Legionella* multiplication (Tables 1-5). Subcultures from the inoculated first day by-products begin to show the multiplication of *Legionella* at the fifth day in case of *Ps. aeruginosa* and *A. baumannii* and at the twelfth day in case of *Prot. mirabilis*. According to the results at the end of sub-culturing period (after 25 days), *Prot. mirabilis* showed superiority in supporting *Legionella* multiplication followed by *Ps. aeruginosa* and finally *A. baumannii* (Tables 1, 2, 4 and 5). The age of bacteria by-products (depend on how long the bacteria stayed in water before membrane filtration) used to represent another factor in determining the ability of the tested species to support *L. pneumophila* multiplication (Tables 1 - 4). To consider this factor in the evaluation of the by-products activity of the four species used, it was supposed that beginning by the second day by-products produced and allover the period of study the species that could give much more samples with higher counts of *Legionella* than the control is the most active one. So, if this evaluation proposal is agreeable, it is possible to arrange the bacterial species used in the following descending order: *Ps. aeruginosa – E. coli – A. baumannii – Prot. mirabilis*. This set of experiments also demonstrated the drop in count of the bacteria including *Legionella* (Table 5) and non-*Legionellaceae* (Tables 1 - 5), when inoculated in sterile tap water, as control, and kept at room temperature. *L. pneumophila* survival was extended to 19 – 25 days (Table 5). In case of other bacteria strains used, the drop in count was followed by a slight increase or stability in numbers (Tables 1 – 5).

DISCUSSION

Legionella is difficult to grow in the laboratory requiring a specific combination of nutrients in the medium. Their nutritional requirements seemed to contradict the widespread distribution of *Legionella* in freshwater environments where nutrient levels are low.

Through this study, a new method was provided for studying the possibility of using bacterial by-products, via a nutritional symbiosis system, for *L. pneumophila* multiplication at room temperature. The four bacterial species used in this study are normally existing in water and especially in the biofilm formed on pipe walls and plumbing system at home, hospitals and network. The possibility of by-products constituents' differences by the time was considered by using the filtrate resulted from membrane filtration for bacteria cultured in sterile tap water as media for culturing *L. Pneumophila* and checking the changes in numbers.

When Yee and Wadowsky [10] demonstrated the possibility of *L. pneumophila* growth in tap water at 37 to 42^{o}C, they mentioned that other investigators [9] were not succeeded to demonstrate this phenomenon through inoculating agar maintained strain of *L. pneumophila* in sterile tap water which incubated at room temperature. In addition, Stout *et al*. [24] findings mentioned to inability of *L. pneumophila* to multiply in a low-nutrient aqueous environment. The present study concentrated specifically on four bacterial species that normally exist in water and associated with biofilm formed on water pipes and plumbing materials that can support bacterial growth. We tried to put the light on the possible of role played by some bacterial species for supporting *L. pneumophila* multiplication in water. Room temperature was used to incubate the seeded sterile tap water in order to simulate the usual conditions in pipes of cold tap water. The results confirmed that bacteria by-products may be different in composition from bacterial species to another and also by time elapsed between inoculation in tap water and membrane filtration to get the growth by-product.

Considering the ability of the four bacterial species to support *L. pneumopila* multiplication, *Prot. mirabilis* by-products of the first day showed the highest function as multiplication supporter, while by the time the by-product showed weak function. The previous character may be due to the inability of *Prot. mirabilis* to grow and the rapid cells viability losses in sterile tap water (Table 2). The data on the ability of *Ps. aeruginosa* as the supporter for multiplication put it in the second position between the four examined bacterial species (Table 4). The superiority of *Ps. aeruginosa* was confirmed by the work of Stout *et al.* [24]. *A. baumannii* comes in the third position as multiplication supporter (Table 3), while *E. coli* by-products showed no effect (Table 1). It was mentioned that *E. coli* isolated from respiratory infection was not able to stimulate *L. pneumophila* growth as satellite colonies when tested in nutritionally deficient agar media [11]. The high multiplication rate that was observed by previous investigators for *legionella* [25-28] may be due to the presence of different microorganisms and slime materials on water pipes that may provide *Legionella* with essential nutrients to proliferate. The first day by-products may contain proteins affected by the extracellular proteases of *L. pneumophila* [11] producing the amino acids needed for supporting the multiplication.

The sequence of the tested bacteria according to their activities from the second day till the 25th day was varied from that appeared for the first day by-products due to the extension in time to show more variation in their by-products composition. The by-products after the 12th days loose most of their abilities to support *Legionella* multiplication which may be due to lowering the metabolic activities as survival strategy of these microorganisms. We would like to attract the attention that the failure in detection the cultured strain of *Legionella pneumophila,* whether in the absence or the presence of other bacteria may be due to its changes, by time, to non-culturable form. Pathogen proliferation potential exists in nearly all water systems. Many factors are involved but most importantly are the presence of microbial biofilms, the degree of microbial diversity, and the availability of nutrients. Managing the microbial fouling process to reduce the risk of Legionnaires' disease principally consists of controlling biofilms and limiting microbial diversity within the entire system. Delineation of the factors which are involved in the multiplication of *L. pneumophila* in aquatic habitats may aid in the formation of practical procedures or protocols necessary for the elimination or prevention of its multiplication in water. Other gram-negative, gram-positive and non-culturable bacterial species which are not included in the present study may have much growth supporting effects and needs much more studies. Some studies should be carried on the chemical composition of bacteria by-products that produced in sterile tap water to but a clear explanation for the differences between species of bacteria as a multiplication or survival supporter for *L. pneumophila*. Special attention should be given to the hospital distribution system as a source of water contamination by other bacteria that can support *Legionella* survival and proliferation. High population number of heterotrophic bacteria in the hospital tap water should be controlled by achieving the free residual chlorine at levels that ensure safety for patients.

T A B L E 1. ***L. pneumophila*** **behaviour as a result of inoculation in** ***E.coli*** **filtrate**

Date / Organism	Changes in *E.coli* and *L.pneumophila* counts (cfu/ml)																				
	26.01	27.01	28.01	29.01	30.01	31.01	01.02	03.02	05.02	07.02	08.02	0.9.02	10.02	12.02	14.02	15.02	16.02	18.02	20.02	22.02	24.02
E.coli	$6.4X10^{8}$	$1.6X10^{6}$	$3.2X10^{6}$	$1.5X10^{5}$	$2.6X10^{5}$	$2.5X10^{5}$	$2.2X10^{5}$	$2.1X10^{6}$	$1.4X10^{5}$	$1.1X10^{5}$	$2.1X10^{5}$		$3.2X10^{5}$	8.0X84	$6.0X10^{4}$		$2.5X10^{4}$	$1.0X10^{4}$	$1.8X10^{3}$	$2.0X10^{3}$	
Filtrate with *Legionella*			$2.0X10^{6}$	$1.2X10^{6}$	$1.4X10^{6}$	$4.6X10^{5}$	$1.3X10^{6}$	$4.0X10^{6}$	$1.1X10^{6}$	$6.0X10^{5}$	$3.9X10^{5}$	$5.5X10^{5}$	$6.8X10^{4}$	$1.3X10^{6}$	1.7X103		$3.0X10^{4}$	$2.0X10^{3}$	<100	<10	
				$1.2X10^{6}$	$1.6X10^{6}$	$3.7X10^{5}$	$1.3X10^{6}$	$8.2X10^{5}$	$8.2X10^{5}$	$4.3X10^{5}$	$4.8X10^{5}$	$4.2X10^{5}$	$6.2X10^{5}$	$4.8X10^{6}$	<100		$1.0X10^{5}$	$1.2X10^{5}$	$3.8X10^{3}$	$5.0X10^{3}$	
					$1.8X10^{6}$	$4.8X10^{5}$	$1.0X10^{6}$	$1.0X10^{6}$	$1.1X10^{6}$	$4.1X10^{5}$	$5.5X10^{5}$	$7.2X10^{5}$	$7.9X10^{5}$	$2.8X10^{6}$	<100		$5.0X10^{5}$	<1000	<100	$5.0X10^{1}$	
						$2.6X10^{4}$	$1.0X10^{4}$	$2.0X10^{4}$	$8.6X10^{3}$	$1.8X10^{4}$	$6.0X10^{5}$	$1.4X10^{6}$	$1.4X10^{6}$	$1.2X10^{6}$	$1.2X10^{6}$		$9.5X10^{5}$	$1.9X10^{6}$	$1.1X10^{6}$	$1.1X10^{6}$	
							$1.0X10^{4}$	$1.0X10^{4}$	$7.2X10^{3}$	$7.1X10^{3}$	$5.1X10^{3}$	$6.2X10^{3}$	$8.4X10^{3}$	$3.1X10^{3}$	$7.0X10^{3}$		<100	<1000	<100	<10	
								<100	$8.0X10^{2}$	$1.0X10^{2}$	$2.0X10^{2}$	$2.0X10^{2}$	$8.0X10^{2}$	$3.1X10^{5}$	$6.0X10^{4}$		$1.2X10^{6}$	$1.1X10^{4}$	$1.1X10^{5}$	$1.1X10^{5}$	
									$1.2X10^{5}$	$8.0X10^{4}$	$7.0X10^{4}$	$2.0X10^{4}$	$3.0X10^{4}$	$1.0X10^{4}$	<100		$1.1X10^{3}$	<100	<100	<10	
										$2.0X10^{4}$	$2.9X10^{4}$	$4.7X10^{4}$	$7.3X10^{5}$	$1.3X10^{6}$	$8.0X10^{5}$		$2.6X10^{3}$	$9.0X10^{3}$	<100	$4.1X10^{2}$	
											$5.8X10^{3}$	$5.6X10^{3}$	$1.8X10^{4}$	$1.6X10^{6}$	$7.8X10^{5}$		$1.0X10^{2}$	<100	<100	<10	
												$2.5X10^{3}$	$2.1X10^{3}$	$1.1X10^{6}$	$1.5X10^{6}$		$9.0X10^{5}$	$7.0X10^{3}$	$8.0X10^{3}$	<10	
													<100	$2.4X10^{3}$	<100		<100		<100	<10	
														$1.2X10^{4}$	<100		$1.8X10^{3}$	$1.0X10^{3}$	<100	<10	
															<100		$1.9X10^{5}$		<100	<10	<10
																$7.2X10^{4}$	$2.6X10^{5}$	<1000	<100	$3.9X10^{4}$	<10
																		<1000	<100	<10	<10
																			$8.1X10^{5}$	$7.8X10^{4}$	$1.6X10^{3}$
																				$2.0X10^{1}$	<10
																					<10

TABLE 2. *L.pneumophila* behaviour as a result of inoculation in *Prot. mirabilis* filtrate

Date / *Organism*	26.01	27.01	28.01	29.01	30.01	31.01	01.02	03.02	05.02	07.02	08.02	0.9.02	10.02	12.02	14.02	15.02	16.02	18.02	20.02	22.02	24.02
	Changes in *Prot. mirabilis* and *L.pneumophila* counts (cfu/ml)																				
P.mirabilis	1.8X10^{8}	5.0X10^{4}	7.0X10^{4}	9.0X10^{4}	5.2X10^{4}	2.5X10^{3}	8.0X10^{2}	3.5X10^{4}	6.1X10^{4}	4.8X10^{4}	1.0X103		6.1X104	<100	<100			<100	<100	<100	
Filtrate with *Legionella*			1.6X10^{6}	9.0X10^{5}	1.3X10^{6}	4.8X10^{5}	1.0X10^{6}	2.0X10^{6}	1.0X10^{6}	4.8X10^{5}	2.1X10^{6}	1.2X106	7.8X10^{5}	4.8X10^{6}	9.6X10^{5}		3.1X10^{5}	1.0X10^{3}	<100	2.8X10^{2}	
				1.3X10^{6}	1.8X10^{6}	3.4X10^{5}	1.1X10^{6}	9.0X10^{5}	1.1X10^{6}	5.3X10^{5}	1.9X10^{6}	1.4X10^{5}	4.8X10^{5}	1.6X10^{6}	1.5X10^{3}		8.0X10^{4}	2.6X10^{4}	1.3X10^{3}	2.2X10^{4}	
					1.7X10^{6}	2.9X10^{5}	7.8X10^{5}	7.1X10^{5}	1.0X10^{6}	3.8X10^{5}	4.3X10^{5}	u.c.	6.8X10^{5}	6.1X10^{6}	<100		4.6X10^{3}	<1000	<100	1.2X10^{2}	
						1.4X10^{4}	1.0X10^{4}	<10^{4}	7.2X10^{3}	1.0X10^{4}	3.0X10^{4}	3.0X10^{4}	9.3X103	2.6X10^{5}	5.0X10^{2}		u.c.	<1000	<100	<10	
							1.0X10^{4}	2.0X10^{4}	8.6X10^{3}	2.8X10^{3}	4.1X10^{3}	3.8X10^{3}		2.1X10^{5}	u.c.		<100	<1000	<100	3.0X10^{10}	
								2.0X10^{4}	6.3X10^{3}	5.8X10^{3}	2.1X10^{3}	2.6X10^{3}	3.6X10^{3}	2.1X10^{6}	1.6X10^{3}		3.0X10^{2}	<100	<100	<10	
									8.0X10^{4}	1.3X10^{4}	6.1X10^{4}	4.0X10^{4}	2.0X10^{4}	1.0X10^{4}	7.0X10^{2}		1.3X10^{3}	<100	<100	<10	
										6.9X10^{3}	1.9X10^{4}	1.7X10^{4}	1.2X10^{6}	2.6X10^{6}	2.0X10^{6}		2.1X10^{3}	3.0X105	<100	<10	
											7.1X10^{3}	7.2X10^{3}	6.9X10^{3}	2.0X10^{6}	1.1X10^{6}		5.0X10^{2}	<100	<100	<10	
												3.1X10^{3}	4.2X10^{3}		<100		3.0X10^{2}	<100	<100	<10	
													9.8X10^{3}	1.0X10^{4}	5.0X10^{2}		<100		<100	<10	
														8.3X10^{3}	100		1.4X10^{3}	1.2X10^{3}	<100	<10	
															<100		2.1X10^{5}		<100	<10	<10
																1.1X10^{5}	9.0X10^{4}	3.0X10^{3}	<100	<10	<10
																		<1000	<100	<10	<10
																				1.1X10^{4}	1.0X10^{3}
																				2.3X10^{3}	<10
																					<10

u.c.: Un countable

TABLE 3. ***L.pneumophila*** **behaviour as a result of inoculation in A** ***baumannii*** **filtrate**

Date / Organism	Changes in A. *baumannii* and *L.pneumophila* counts (cfu/ml)																				
	26.01	27.01	28.01	29.01	30.01	31.01	01.02	03.02	05.02	07.02	08.02	09.02	10.02	12.02	14.02	15.02	16.02	18.02	20.02	22.02	24.02
A.baumannii	$4.9X10^{7}$	$4.0X10^{4}$	$1.9X10^{6}$	$2.6X10^{5}$	$2.8X10^{5}$	$4.4X10^{5}$	$5.0X10^{5}$	$1.5X10^{5}$	$7.8X10^{4}$	$5.4X10^{4}$	$1.8X10^{5}$		$9.0X10^{4}$	$6.5X10^{4}$	$1.1X10^{5}$		$1.1X10^{5}$	$2.0X10^{4}$	$1.4X10^{3}$	$2.1X10^{4}$	
Filtrate with *Legionella*			$1.7X10^{6}$	$1.5X10^{6}$	$2.0X10^{6}$	$5.1X10^{5}$	$1.2X10^{6}$	$1.0X10^{6}$	$1.2X10^{6}$	$6.3X10^{5}$	$1.9X10^{6}$	$5.2X10^{5}$	$6.3X10^{5}$	$2.4X10^{6}$	$7.8X10^{3}$		$7.0X10^{4}$	$3.0X10^{3}$	$4.0X10^{2}$	$2.0X10^{1}$	
				$1.1X10^{6}$	$1.9X10^{6}$	$3.1X10^{6}$	$1.3X10^{6}$	$1.2X10^{6}$	$1.0X10^{6}$	$7.0X10^{5}$	$7.3X10^{5}$	$4.1X10^{5}$	$5.8X10^{5}$	$2.8X10^{6}$	$3.4X10^{3}$		u.c.	$2.5X10^{4}$	4.0X85	$4.3X10^{4}$	
					$1.7X10^{6}$	$3.9X10^{5}$	$7.2X10^{5}$	$9.1X10^{5}$	$1.2X10^{6}$	$3.5X10^{5}$	$5.1X10^{5}$	$6.1X10^{5}$	$9.1X10^{5}$	$5.2X10^{6}$	$1.9X10^{3}$		$5.9X10^{5}$	$7.0X10^{5}$	<100	$8.5X10^{2}$	
						$2.3X10^{4}$	$3.0X10^{4}$	$1.0X10^{4}$	$1.4X10^{4}$	$1.2X10^{4}$	$3.8X10^{3}$	$6.0X10^{3}$	$4.3X10^{5}$	$6.7X10^{5}$	< 100		$2.0X10^{3}$	<1000	<100	<10	
							$1.0X10^{5}$	$3.0X10^{4}$	$8.5X10^{3}$	$6.5X10^{3}$	$4.7X10^{3}$	$4.4X10^{3}$		$3.2X10^{3}$	<100		$2.0X10^{2}$	<1000	<100	<10	
								$3.0X10^{4}$	$7.1X10^{3}$	$5.1X10^{3}$	$8.3X10^{3}$	$4.8X10^{3}$	$5.1X10^{3}$	$3.2X10^{5}$	$9.0X10^{4}$		$1.1X10^{3}$	<100	<100	<10	
									$3.1X10^{5}$	$9.0X10^{3}$	$1.6X10^{4}$	$2.0X10^{4}$	$3.0X10^{4}$	$1.1X10^{4}$	$1.0X10^{2}$		$7.0X10^{2}$	<100	<100	<10	
										$4.0X10^{4}$	$7.1X10^{5}$	$1.9X10^{5}$	$7.5X10^{5}$	$1.4X10^{6}$	$5.0X10^{5}$		$2.8X10^{4}$	$5.0X10^{3}$	$7.1X10^{4}$	$2.9X10^{4}$	
											$8.1X10^{3}$	$6.4X10^{3}$	$4.8X10^{5}$	$1.1X10^{6}$	$1.0X10^{6}$		$1.1X10^{3}$	$1.0X10^{3}$	<100	<10	
												$2.7X10^{3}$	$2.2X10^{3}$	$1.8X10^{3}$	$1.0X10^{2}$		$4.0X10^{2}$	<100	<100	<10	
													$9.2X10^{3}$	$1.0X10^{4}$	<100		$1.0X10^{3}$		<100	<10	
														$9.0X10^{3}$	$1.0X10^{2}$		$3.1X10^{3}$	$1.0X10^{3}$	<100	<10	
															<100		u.c		<100	<10	<10
																$4.2X10^{5}$	$4.2X10^{5}$	<1000	<100	<10	<10
																		<1000	<100	<10	<10
																			<100	<10	<10
																				<10	<10
																					<10

u.c.: Un countable

TABLE 4. ***L.pneumophila*** **behaviour as a result of inoculation in** ***P.aeruginosa*** **filtrate**

Date / Organism	Changes in *P.aeruginosa* and *L.pneumophila* counts (cfu/ml)																				
	26.01	27.01	28.01	29.01	30.01	31.01	01.02	03.02	05.02	07.02	08.02	0.9.02	10.02	12.02	14.02	15.02	16.02	18.02	20.02	22.02	24.02
P.aeruginosa	$2.3X10^{9}$	$2.0X10^{6}$	$1.1X10^{7}$	$6.1X10^{7}$	$5.4X10^{6}$	$5.8X10^{6}$	$5.2X10^{6}$	$1.2X10^{7}$	$3.7X10^{6}$	$4.1X10^{6}$	$1.5X10^{7}$		$2.1X10^{6}$	$2.1X10^{6}$	$1.2X10^{6}$		$1.4X10^{6}$	$2.1X10^{6}$	$1.0X10^{6}$	$1.1X10^{6}$	
Filtrate with *Legionella*			$1.8X10^{6}$	$1.5X10^{6}$	$1.1X10^{6}$	$3.8X10^{5}$	$1.4X10^{6}$	$1.0X10^{6}$	$1.2X10^{6}$	$6.1X10^{5}$	$4.8X10^{5}$	$6.8X10^{5}$	$5.1X10^{5}$	u.c.	$1.2X10^{4}$		$2.4X10^{5}$	$2.1X10^{4}$	<100	$2.0X10^{3}$	
				$1.0X10^{6}$	$1.7X10^{6}$	$4.5X10^{5}$	$4.0X10^{6}$	$1.1X10^{6}$	$9.0X10^{5}$	$5.3X10^{5}$	$1.1X10^{6}$	$1.8X10^{5}$	$5.1X10^{5}$	$1.9X10^{6}$	$1.0X10^{4}$		$1.4X10^{5}$	$1.0X10^{3}$	$4.0X10^{4}$	$2.0X10^{4}$	
					$1.9X10^{6}$	$4.6X10^{5}$	$1.0X10^{6}$	$9.3X10^{5}$	$7.4X10^{5}$	$5.6X10^{5}$	$4.1X10^{5}$	$4.4X10^{5}$	$6.1X10^{5}$	$1.6X10^{6}$	$2.9X10^{3}$		$2.6X10^{5}$	<1000	$9.0X10^{3}$	$2.5X10^{3}$	
						$2.1X10^{4}$	$2.0X10^{4}$	$1.0X10^{4}$	$8.5X10^{3}$	$1.3X10^{4}$	$1.8X10^{5}$	$1.2X10^{5}$	$5.1X10^{5}$	$4.8X8^{5}$	$7.6X10^{5}$		$1.6X10^{6}$	$3.8X10^{6}$	$2.1X10^{6}$	$2.2X10^{6}$	
							$7.0X10^{4}$	$4.0X10^{4}$	$8.1X10^{3}$	$4.1X10^{3}$	$4.9X10^{3}$	$1.1X10^{4}$		u.c.	<100		$2.0X10^{2}$	<1000	<100	<10	
								$2.0X10^{4}$	$1.0X10^{3}$	$7.6X10^{3}$	$6.3X10^{3}$	$8.1X10^{3}$	$5.9X10^{3}$	$6.8X10^{1}$	$6.0X10^{3}$		$1.8X10^{3}$	<100	<100	<10	
									$1.3X10^{5}$	$9.0X10^{4}$	$8.2X10^{4}$	u.c.	$3.0X10^{4}$	u.c.	<100		$1.2X10^{3}$	<100	<100	$8.3X10^{2}$	
										$4.0X10^{4}$	u. c.	u.c.	$2.2X10^{5}$	$3.0X10^{5}$	$4.0X10^{5}$		$4.9X10^{3}$	$2.0X10^{5}$	$8.0X10^{4}$	$1.1X10^{5}$	
											$5.8X10^{3}$	$6.9X10^{3}$	$5.2X10^{3}$	$2.3X10^{6}$	$1.6X10^{6}$		$3.9X10^{3}$	$1.0X10^{3}$	<100	$8.0X10^{2}$	
												$2.4X10^{3}$	$3.3X10^{3}$	$1.8X10^{3}$	<100		<100	<100	<100	<10	
													$1.3X10^{3}$	$1.9X10^{4}$	<100		<100		<100	<10	
														$1.5X10^{6}$	$1.8X10^{4}$		$4.0X10^{4}$	$9.0X10^{4}$	$7.0X10^{4}$	$9.8X10^{4}$	
															<100		$2.4X10^{5}$		<100	<10	<10
																$3.8X10^{4}$	$1.6X10^{5}$	$2.0X10^{3}$	<100	$3.0X10^{1}$	<10
																		$1.3X10^{4}$	$4.0X10^{5}$	<10	$1.8X10^{4}$
																			$3.0X10^{3}$	<10	<10
																				$7.0X10^{3}$	<10
																					<10

u.c.: Un countable

TABLE 5. Survival of *L.pneumophila in* sterile drinking water

Date / Organism	Changes in *L.Pneumophila* counts (cfu/ml)																				
	26.01	27.01	28.01	29.01	30.01	31.01	01.02	03.02	05.02	07.02	08.02	09.02	10.02	12.02	14.02	15.02	16.02	18.02	20.02	22.02	24.02
Legionella		$1.4X10^{6}$	$7.9X10^{5}$	$1.3X10^{6}$	$2.2X10^{6}$	$5.2X10^{5}$	$1.8X10^{6}$	$1.4X10^{3}$	$1.2X10^{6}$	$5.0X10^{5}$	$6.5X10^{5}$	$8.5X10^{5}$	$6.1X10^{5}$	$5.2X10^{5}$	$6.0X10^{3}$		$2.0X10^{4}$	$1.0X10^{3}$	$1.0X10^{3}$	u.c.	
in sterilized			$5.2X10^{6}$	$1.6X10^{6}$	$2.7X10^{6}$	$6.5X10^{5}$	$1.1X10^{6}$	$3.1X10^{6}$	$1.3X10^{6}$	$5.3X10^{5}$	$2.5X10^{6}$	$6.7X10^{5}$	$7.3X10^{5}$	$1.5X10^{6}$	$7.1X10^{3}$		$3.1X10^{5}$	$8.0X10^{3}$	$4.0X10^{2}$	<10	
drinking				$2.4X10^{6}$	$2.1X10^{6}$	$6.1X10^{5}$	$1.4X10^{6}$	$1.2X10^{6}$	$1.1X10^{6}$	$6.5X10^{5}$	$3.8X10^{5}$	$3.2X10^{5}$	$2.1X10^{6}$	U.C.	$1.5X10^{4}$		$5.2X10^{5}$	$6.5X10^{4}$	$9.0X10^{4}$	u.c.	
water					$2.0X10^{4}$	$1.5X10^{4}$	$4.0X10^{4}$	$1.0X10^{4}$	$7.9X10^{3}$	$2.0X10^{4}$	$4.9X10^{5}$	$5.1X10^{5}$	$7.8X10^{5}$	$1.1X10^{6}$	$9.0X10^{4}$		$7.0X10^{4}$	$2.8X10^{4}$	$4.5X10^{3}$	<10	
						$8.6X10^{5}$	$2.9X10^{4}$	$6.0X10^{4}$	$7.2X10^{3}$	$1.2X10^{5}$	$2.8X10^{4}$	$4.0X10^{4}$	$1.8X10^{4}$	$2.0X10^{4}$	$7.0X10^{3}$		$1.1X10^{5}$	$3.0X10^{5}$	$3.2X10^{5}$	$3.0X10^{5}$	
							$4.0X10^{4}$	$3.2X10^{4}$	$7.3X10^{3}$	$4.8X10^{3}$	$2.6X10^{3}$	$2.8X10^{3}$	$2.6X10^{3}$	$2.0X10^{4}$	$4.1X10^{4}$		$7.0X10^{2}$	<100	<100	<10	
								$3.3X10^{5}$	$1.9X10^{5}$	$9.0X10^{4}$	$6.0X10^{4}$	$3.8X10^{4}$	$1.0X10^{4}$	$2.0X10^{4}$	$7.5X10^{3}$		$2.0X10^{4}$	<100	<100	<10	
									$4.0X10^{4}$	$7.9X10^{3}$	$4.3X10^{3}$	$3.8X10^{4}$	$6.1X10^{5}$	$8.3X10^{5}$	$8.8X10^{5}$		$5.2X10^{3}$	$4.1X10^{4}$	<100	<10	
										$1.1X10^{4}$	$5.3X10^{3}$	$6.0X10^{3}$	$6.5X10^{4}$	$1.5X10^{6}$	$9.2X10^{5}$		<100	<100	<100	<10	
											$3.1X10^{3}$	$3.0X10^{3}$	$2.6X10^{3}$	$1.2X10^{3}$	<100		<100	<100	<100	<10	
												u.c.	$5.0X10^{4}$	$9.0X10^{4}$	$1.9X10^{4}$		$6.0X10^{4}$		<100	$3.2X10^{2}$	
													$9.0X10^{4}$	$3.0X10^{5}$	$2.1X10^{5}$		$7.0X10^{4}$	$1.3X10^{3}$	<100	<10	
														$1.2X10^{6}$	$4.0X10^{4}$		$8.9X10^{5}$	$1.0X10^{3}$	<100	<10	<10
															$8.1X10^{5}$	$1.2X10^{5}$	$1.2X10^{5}$	<1000	<100	<10	<10
																	$2.1X10^{5}$	$3.0X10^{3}$	<100	<10	$3.1X10^{3}$
																		$1.1X10^{5}$	$4.6X10^{4}$	$1.0X10^{4}$	$1.1X10^{4}$
																				$5.0X10^{3}$	<10
																					<10

u.c. : Un countable

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