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Final Screening Assessment for Enterobacter aerogenes strain ATCC 13048

Environment and Climate Change Canada

Health Canada

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Canada

Synopsis

Pursuant to paragraph 74(b) of the Canadian Environmental Protection Act, 1999 (CEPA), the Minister of the Environment and the Minister of Health have conducted a screening assessment of Enterobacter aerogenes strain ATCC[1] 13048.

E. aerogenes strain ATCC 13048 is a bacterium that has characteristics in common with other strains of the species Enterobacter aerogenes. The genus Enterobacter is widespread in nature, with species present in marine and fresh water, in sewage and soil, and on plants. E. aerogenes is part of the normal flora of the human and animal gastrointestinal tract and is also found on the mucosal surfaces of animals.
E. aerogenes strains grow over a wide range of temperatures and pH values.
E. aerogenes has characteristics that make it of interest for a variety of applications, including water and wastewater treatment, bioremediation, production of energy and fuels and enzyme production.

E. aerogenes is a well-known organism which can, under certain conditions, infect some animals and cause a range of symptoms that can debilitate the host and even kill it. Under normal circumstances it is unlikely to be a serious hazard to healthy livestock or other organisms in the environment. E. aerogenes can cause mastitis in cows, but affected animals recover rapidly upon treatment with veterinary antibiotics. Some invertebrates are susceptible to E. aerogenes. Despite its prevalence and association with various environmental species and habitats, there is no evidence in the scientific literature to suggest that E. aerogenes has any adverse ecological effects at the population level for plants, vertebrates or invertebrates.

In humans, E. aerogenes is a nosocomial (hospital-acquired) pathogen with the potential to cause a wide variety of infections, including wound infections, meningitis, respiratory and urinary tract infections, bacteremia, septicemia and septic shock.
E. aerogenes infections are generally associated with antibiotic treatment, medical devices, long hospital stays and immunosuppression. Although strain ATCC 13048 is susceptible to many antibiotics, E. aerogenes as a species is well known for its ability to develop resistance to antibiotics of various classes that are of very high importance to human medicine. Development of resistance in strain ATCC 13048 could compromise the effectiveness of treatments for infection with this strain.

This assessment considers the aforementioned characteristics of E. aerogenes strain ATCC 13048 with respect to environmental and human health effects associated with consumer and commercial product use and industrial processes subject to CEPA, including releases to the environment through waste streams and incidental human exposure through environmental media. To update information about current uses, the Government launched a mandatory information-gathering survey under section 71 of CEPA, as published in the Canada Gazette, Part I on

[1] American type culture collection

October 3, 2009 . Information submitted in response to the section 71 notice indicates that E. aerogenes strain ATCC 13048 was not imported into or manufactured in Canada in 2008, except in limited quantities for research and development as well as teaching activities.

Based on the information available, it is concluded that E. aerogenes strain ATCC 13048 does not meet the criteria under paragraph 64(a) or (b) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

It is concluded that E. aerogenes strain ATCC 13048 does not meet the criteria under paragraph 64(c) of CEPA as it is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

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Introduction

Pursuant to paragraph 74(b) of the Canadian Environmental Protection Act, 1999 (CEPA), the Minister of the Environment and Climate Change and the Minister of Health are required to conduct screening assessments of living organisms added to the Domestic Substances List (DSL) by virtue of section 105 of the Act to determine whether they present or may present a risk to the environment or human health (according to criteria as set out in section 64 of CEPA) [2]. E. aerogenes strain ATCC 13048 was added to the DSL under subsection 105(1) of CEPA because it was manufactured in or imported into Canada between January 1, 1984 and December 31, 1986 and it entered or was released into the environment without being subject to conditions under CEPA or any other federal or provincial legislation.

This screening assessment considers hazard information obtained from the public domain and from unpublished research data generated by Health Canada[3] and Environment and Climate Change Canada[4] research scientists as well as comment from scientific peer reviewers. Exposure information was also obtained from the public domain as well as from a mandatory CEPA section 71 notice published in the Canada Gazette, Part I, on October 3, 2009. Further details on the risk assessment methodology used are available in the "Framework on the Science-Based Risk Assessment of Micro-organisms under the Canadian Environmental Protection Act, 1999" (Environment Canada and Health Canada 2011).

In this report, data that are specific to DSL-listed strain E. aerogenes strain ATCC 13048 are identified as such. Where strain-specific data were not available, surrogate information from literature searches was used. When applicable, literature searches conducted on the organism included its synonyms, and common and superseded names. Surrogate organisms are identified in each case to the taxonomic level provided by the source. Literature searches were conducted using scientific literature databases (SCOPUS, Google Scholar, CAB abstracts), web searches and key search terms for the identification of human health and environmental hazards of the DSL strain assessed in this report. Information identified up to April 2017 was considered for inclusion in this report.

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[2] A determination of whether one or more criteria of section 64 of CEPA are met is based on an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA may not be relevant to, nor does it preclude, an assessment against the criteria specified in the Hazardous Products Regulations, which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.

[3] Testing conducted by Health Canada's Environmental Health Science and Research Bureau

[4] Testing conducted by Environment and Climate Change Canada's Ecotoxicology and Wildlife Health Division

Decisions from domestic and international jurisdictions

Domestic

E. aerogenes is a Risk Group 2 human and animal pathogen and as such, it is regulated by the Public Health Agency of Canada and by the Canadian Food Inspection Angency. They are regulated under the Human Pathogens and Toxins Act and their use in research and teaching laboratories should be in compliance with the Canadian Biosafety Standard Second Edition, 2015 (CBS 2015).

Enterobacter species and Enterobacter aerogenes are listed in the Transportation of Dangerous Goods Regulations as a Category B infectious substance. Arrangements for shipping of E. aerogenes strain ATCC 13048 must also meet requirements under Canada's Transportation of Dangerous Goods Act and Regulation. These measures are designed to prevent any human or environmental exposure to the micro-organisms during transport. Human and environmental exposure to E. aerogenes strain ATCC 13048 through R&D and teaching uses reported under the notice are therefore expected to be low.

International

E. aerogenes has been investigated as a biological pesticide; however, no strains are currently registered under the Pest Control Products Act or the Federal Insecticide, Fungicide, and Rodenticide Act administered by the United States Environmental Protection Agency. It has been included in the United States Food and Drug Administration Bad Bug Book: Handbook for Foodborne Pathogenic Micro-orgnaisms ad Natureal Toxins due to its opportunistic pathogenicity and its isolation from food sources. Enterobacter aerogenes is also listed as a regulated livestock and poultry pathogen by the United States Animal and Plant Health Inspection Services. No other international decisions regarding Enterobacter aerogenes were found[5].

[5] Government agencies and organizations searched include: World Health Organization; United States Centers for Disease Control; Biosecurity NZ; Australian Department of Health; European Food Safety Authority; and the European Centre for Disease Prevention and Control.

1. Hazard assessment

1.1 Characterization of Enterobacter aerogenes

1.1.1 Taxonomic identification and strain history

Binomial name: Enterobacter aerogenes

Kingdom:	Bacteria
Phylum:	Proteobacteria
Class:	Gammaproteobacteria
Order:	Enterobacteriales
Family:	Enterobacteriaceae
Genus:	Enterobacter
Species:	Enterobacter aerogenes
DSL strain:	ATCC 13048

Synonyms, common and superseded names:

The classification of E. aerogenes has evolved since the deposition of strain ATCC 13048. The genus Aerobacter was replaced by Enterobacter to resolve confusion arising from the reclassification of many motile A. aerogenes strains (but not strain ATCC 13048) to the genus Klebsiella (Hormaeche and Edwards 1960 a and b). Many advocate the transfer of E. aerogenes to the Klebsiella genus and the use of Klebsiella mobilis instead of E. aerogenes (Skerman et al. 1980) based on numerical taxonomy (Bascom et al. 1971). Phylogenetic analyses of Enterobacteriaceae based on 16S ribosomal RNA (rRNA) gene sequences and gyrB sequence alignments (Boye and Hansen 2003; Dauga 2002; Drancourt et al. 2001), MALDI-TOF Biotyper analysis and in silico DNA-DNA hybridization (Diene et al. 2013) showed a higher similarity between E. aerogenes and members of the genus Klebsiella than between E. aerogenes and other members of the genus Enterobacter. Therefore, in this risk assessment and as presented in the Bergey's manual (Grimont and Grimont 2005), information on the genera Enterobacter and Klebsiella and on the species E. aerogenes and K. mobilis will be considered.

E. aerogenes strain ATCC 13048 is the type strain of the species and has several homologous strain numbers in other culture collections (Table 1-1).

Table 1-1: Homologous strains to E. aerogenes strain ATCC 13048 in other culture collections

Culture collection	Accession number
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH	DMS 30053
Japan Collection of Micro-organisms	JCM 1235
National Collection of Type Cultures	NCTC 10006

Strain history:

E. aerogenes strain ATCC 13048 was isolated from a sputum sample by the South Carolina Department of Health and Environmental Control (USA). It was originally deposited to the American Type Culture Collection (ATCC) as Aerobacter aerogenes.

1.1.1.1 Phenotypic characteristics

E. aerogenes is a Gram-negative, motile, straight rod, non-spore forming bacterium. Colonies are generally circular, raised and moist with an entire margin and vary from beige to off-white.

Standard media for the isolation of Entrobacteriaceae can be used to grow E. aerogenes but are not differential for E. aerogenes. On Eosin Methylene Blue (EMB) agar, E. aerogenes produces large, mucoid, pink to purple colonies like Klebsiella species and other Enterobacter species On MacConkey agar, Enterobacter species and Klebsiella species produce pink colonies. A combination of these media with simple biochemical tests is efficient in detecting and identifying E. aerogenes (see Table 1-2 and Table 1-3).

Table 1-2: Comparison of biochemical characteristics of E. aerogenes and related Enterobacteriaceae species

Characteristic	E. aerogenes strain ATCC 13048 [a]	E. aerogenes/ K. mobilis[b]	Klebsiella pneumonia[b]	Klebsiella terrigena[b]	Enterobacter cloacae complex[b]
Motility	+	+	-	-	v
Gluconate dehydrogenase	+	+	v	+	-
Lysine decarboxylase	+	+	v	+	-
Ornitine decarboxylase	+	+	-	-	+
Urea hydrolyse	-	-	v	+	-

+ indicates positive; - indicates negative; v indicates variation between strains
[a] Test conducted by Environmental Health Science and Research Bureau
[b] Adapted from Bergey's Manual of Systematic Bacteriology (Grimont and Grimont 2005)

Table 1-3: Comparison of carbohydrate utilisation profiles of E. aerogenes and related Enterobacteriaceae

Carbohydrate	E. aerogenes strain ATCC 13048 [a]	E. aerogenes/ K. mobilis[b]	Klebsiella pneumonia[b]	Klebsiella terrigena[b]	Enterobacter cloacae complex[b]
L-Arabitol	-	-	-	-	+
Benzoate	N/A	+	v	v	-
Histamine	N/A	+	-	v	-
3-hydroxybenzoate	N/A	+	-	+	-
Protocatechuate	N/A	+	v	+	-
D-Sorbose	+	-	+	+	N/A
Quinate	N/A	+	v	+	-
Tricarballylate	N/A	+	v	+	-

+ Indicates positive; - indicates negative; v indicates variation between strains; N/A indicates data not available
[a] Test conducted by Environmental Health Science and Research Bureau
[b.] Adapted from Bergey's Manual of Systematic Bacteriology (Grimont and Grimont 2005)

Analysis of phenotypic characteristics is essential for proper identification of E. aerogenes and distinction from closely related enterobacteria. Carbon utilisation profiles are essential in the differentiation of Klebsiella, including K. mobilis/E. aerogenes from other enterobacteriaceae. All Klebsiella, with the exception of K. pneumoniea subsp. ozaenae and subsp. rhinascleromatis, use D-arabitol, myo-inositol, palatinose, quinate, D-sorbitol and sucrose. There are no species of Enterobacter, Pantoea or Erwinia that are able to use all six substances with the exception of E. aerogenes. No Serratia species are able to use all six substances simultaneously except for S. ficaria, and S. ficaria can be differentiated from Klebsiella due to its use of L-arabitol and i-erythriol (Grimont and Grimont 2005).

1.1.1.2 Molecular characteristics

Phylogenetic approaches based on 16S rRNA gene sequences provide limited resolution for enterobacterial genus and species identification. For example, in confirmatory testing at Health Canada[6], 16S rRNA gene sequence analyses of E. aerogenes strain ATCC 13048 showed greater than 99% sequence identity (less than 4 base pairs difference) with the ATCC 13048 sequence in the proprietary MicroSeq ® ID library and high sequence identity to ATCC 13048 in NCBI, but also matched to other Klebsiella and Kluyvera species at ≥97% homology in MicroSeq® ID and to members of the following enterobacterial genera : Kluyvera, Raoultella, Klebsiella, Enterobacter, Citrobacter, Serratia, Escherichia, Bittuauxella, Salmonella, Pectobacterium, Hafnia, Cronobacter, Tatumella, Erwinia, Shigella, Pantoea, Yersinia in NCBI. A few PCR methods for rapid detection and identification of important pathogens have been developed but have a questionable sensitivity and accuracy when applied to E. aerogenes (Lehmann et al. 2008).

The entire genome of E. aerogenes strain ATCC 13048 (under the designation KCTC 2190) has been sequenced (accession number CP002824). It has a G+C content of 54.8%. The genome consists of a single circular 5.28 Mb chromosome,

[6] Environmental Health Science and Research Bureau

which includes 4,912 coding genes (77.85% with putative functions assigned by annotation) (Shin et al. 2012). The genome of a multidrug-resistant E. aerogenes strain has also been sequenced. It has a G+C content of 55% and includes a chromosome of 5.42 Mb and two plasmids (162.2 Kb and 9.2 Kb) (Diene et al. 2013).

1.1.2 Biological and ecological properties

1.1.2.1 Natural occurrence

Enterobacter species are ubiquitous and are found in marine and fresh water, sewage, soil and plants (Chan and Kueh 1976; Leclerc et al. 2001; Marchi and Utkhede 1994; Richard 1963; Werner et al. 1974).

E. aerogenes has been isolated from a wide range of terrestrial and aquatic vertebrates (Goda et al. 1986; Hoda et al. 1993; Okaeme, 1989; Old et al. 1998; Platt et al. 1976; Venugopal et al. 1980; Verma et al. 1982; Wakwoya, 2006). They are also found on the mucosal surface of animals and E. aerogenes is part of the normal flora of the human gastro-intestinal tract and a skin resident (Adris 2006; Chow et al. 1991; Chevalier et al. 1999; Cosgrove et al. 2002; Fawcett et al. 1986; Richard 1963). Pathogenic aerobic bacteria isolated from 150 hen egg shells and egg contents were identified (Fardows and Shamuzzaman 2015). E. aerogenes was isolated from 10 egg shells but not the contents.

1.1.2.2 Survival, persistence and dispersal in the environment

In persistence studies conducted by Environment Canada[7], DNA from E. aerogenes strain ATCC 13048 was recovered from sandy loam agricultural soil in the laboratory up to 125 days after inoculation of the soil with viable cells, but not at 180 days post-inoculation (Xiang et al. 2010). It was not determined if the extracted DNA was from viable or dead cells.

In seawater, survival of E. aerogenes is limited by high pH (≥ 7.5) (Chan et al. 1979) and salinity greater than 3% (Chan and Kueh et al. 1976). It has the capacity to survive in environments where year-round temperatures remain near 0°C (Emde et al. 1992), suggesting that it could survive Canadian winters at least in some parts of the country.

In soil and marine ecosystems, E. aerogenes is often the favoured prey of various bacteria but it manages to maintain a presence throughout these environments (Andersen et al. 2004; Gupta and Germida 1989).

Overall, given the ubiquity of the species, it is likely that this strain is able to survive for considerable lengths of time in soil and other media even if there is no evidence of proliferation.

[7] Biological Assessment and Standardization Section, Soil Biotechnology Laboratory

1.1.2.3 Growth parameters

The growth of E. aerogenes strain ATCC 13048 in various liquid and solid media at temperatures between 28°C and 42°C is described in Appendix 1. E. aerogenes isolated from environmental sources grows optimally between 20°C and 30°C and at 18°C when grown in sterilized soil; clinical isolates grow optimally at 37°C (Gupta et al. 1986; Richard 1963).

The species is a facultative anaerobe known to produce hydrogen. It is able to perform N_2 fixation as well as fermentation of various sugars including: lactose, dextrose, sucrose, galactose, xylose, arabinose, mannose, and rhamnose (Converti et al. 2002; Goyal et al. 2013; Richard, 1963; Werner et al. 1974).

1.1.2.4 Role in nutrient cycling

The metabolic activity of E. aerogenes strain NCIM 2304 varies with oxygen availability and E. aerogenes, as a species, is sensitive to changes in salinity as higher salinity leads to reduced N_2 fixating capabilities (Mukherjee et al. 1999; Werner et al. 1974).

1.1.2.5 Resistance to antibiotics, metals and chemical agents

The antibiotic susceptibility profile of E. aerogenes strain ATCC 13048 found in the literature and performed by scientists at Health Canada[8] (see Table 1-4) shows that E. aerogenes strain ATCC 13048 is susceptible to many antibiotic agents and has not acquired resistance to antibiotics of importance to human medicine, including 3rd and 4th generation cephalosporins, carbapenems or quinolones.

Table 1-4: Minimal Inhibitory concentration (MIC, µg/ml) for E. aerogenes strain ATCC 13048

Antibiotic	MIC breakpoint[a] (µg/ml)	HC[b]	Gayet et al. 2003[c]	Chen et al. 2008a[d]	Martins et al. 2010[e]	Interpretation	Antibiotic Importance[f]
Amikacin	S≤16; I 32; R≥64	N/A	4	0.5	N/A	S	High
Amoxycillin	S≤8; ; I 16; R≥32	>24	N/A	N/A	N/A	R	High
Aztreonam	S≤4; I 8; R≥16	1.8 +/- 2.5	1	N/A	N/A	S	Very high
Cefepime (4th gen cephalosporin)	S≤2; I 4-8; R≥16	N/A	N/A	0.064	0.125	S	Very high
Cefotaxime (3rd gen. cephalosporin)	S≤1; I 2; R≥4	2.1 +/- 2.7	0.5	0.25	N/A	S	Very high
Ceftazidime (3rd gen. cephalosporin)	S≤4; I 8; R≥16	0.75	1	0.5	0.5	S	Very high
Cefoperazone (3rd gen. cephalosporin)	S≤16; I 32; R≥64	N/A	N/A	0.25	N/A	S	Very high

[8] Environmental Health Science and Research Bureau

Antibiotic	MIC breakpoint[a] (µg/ml)	HC[b]	Gayet et al. 2003[c]	Chen et al. 2008a[d]	Martins et al. 2010[e]	Interpretation	Antibiotic Importance[f]
Chloramphenicol	S≤8; I 16; R≥32	N/A	16	N/A	8	I	Medium
Ciprofloxacin	S≤1; I 2; R≥4	0.38	0.125	N/A	0.125	S	Very high
Colistin	N/A	1.9+/- 2.1	N/A	N/A	N/A	N/A	Very high
Erythromycin	N/A	>24	N/A	N/A	128	N/A	High
Gentamicin	S≤4; I 8; R≥16	4.2 +/- 1.6	1	0.5	1	S	High
Imipemen	S≤1; I 2; R≥4	N/A	0.125	0.125	0.25	S	Very high
Meropenem	S≤1; I 2; R≥4	0.38	N/A	0.064	N/A	S	Very high
Nalidixic Acid	S≤16; R≥32	20 +/- 6.9	4	N/A	8	S-I	Very high
Norfloxacin	S≤4l I 8; R≥16	N/A	0.5	N/A	0.5	S	Very high
Polymixin B	N/A	N/A	N/A	1	N/A	N/A	Very high
Tetracycline	S≤4; I 8; R≥16	N/A	2	N/A	2	S	Medium
Trimethoprim	S≤8; R≥16	>24	N/A	N/A	N/A	R	Medium

N/A indicates that data is not available; S indicates susceptible; I indicates intermediate; R indicates resistant

[a] Breakpoints to determine the susceptibility of the strain were taken from Clinical and Laboratory Standard Institute's Performance Standard for Antibiotic Susceptibility testing

[b] Test conducted using the TSB-MTT liquid assay method (Seligy et al. 1997). The reported values are based on a minimum of four independent experiments. Values correspond to the minimal inhibitory concentration (µg/ml) and their standard deviation for the select E. aerogenes strain (20,000 colony forming units /well) incubated in the presence of antibiotic for 24 hrs at 37°C.

[c] MICs performed as per Clinical and Laboratory Standard Institute Guideline for microdilution method

[d] MICs determined using Etest strips (AB BIODISK, Solna, Sweden)

[e] MICs performed as per Clinical and Laboratory Standard Institute Guideline for microdilution method

[f] Based on Categorization of "Antimicrobial Drugs Based on Importance in Human Medicine" document produced by Veterenary Drug directorate of Health Canada

E. aerogenes is well known for its ability to acquire resistance to antibiotics used against enterobacterial infections. This occurs through the activation or inactivation of chromosomal genes or through the horizontal acquisition of new genes and is generally associated with the use of antibiotics (Barnaud et al. 2004; Jacobson et al.1995; Lee et al. 2002; Pfaller et al. 1997). Previously susceptible E. aerogenes strains can acquire or develop a resistant phenotype in less than a week (Bornet et al. 2000).

E. aerogenes is naturally resistant to amoxicillin, amoxicillin-clavunic acid combination and first- and second-generation cephalosporins due to the expression of a chromosomal inducible Bush-Jacoby-Medeiros group 1 β-lactamase, ampC. Resistance can increase following a mutation in the chromosomal gene ampD that normally prevents high expression of the chromosomal β-lactamase and by induction by some β-lactam substrates (Sanders 1992; Sander and Sander 1997). Mutation of the ampC gene can broaden activity by conferring resistance to carbapenems and all cephalosporinases (including 4th generation). Such a mutant was isolated from a patient treated with ceftriaxone and cefepime (Fernandez-Cuenca et al. 2006; Rodriguez-Martinez et al. 2012). E. aerogenes strains carrying derepressed chromosomal β-lactamase (resistant to 3rd generation cephalosporinases) have been implicated in hospital-associated outbreaks (see Appendix 2), infections (Chow et al. 1991), and the development of this type of resistance is associated with significant adverse outcomes (Cosgrove et al. 2002).

The capacity of E. aerogenes to rapidly develop a multidrug resistant (MDR) phenotype is associated with changes in the regulation, expression and function of porins, membrane transporters and efflux pumps (Chen et al. 2008a; Davin-Regli et al. 2008; Martins et al. 2010). Porin channel alterations that reduce the influx of antibiotics such as β-lactams, imipenem, or carbapenems have been associated with resistance in clinical isolates (Bornet et al. 2000; Chen et al. 2008a; Chevalier et al. 1999; Mallea et al. 1998; Thiolas et al. 2004; Tzouvelekis et al. 1994; Yigit et al. 2002). Efflux pumps actively export antibiotics before they can reach their target in the cell. Over expression of AcrAB-TolC and EefABC efflux pumps have been implicated in antibiotic resistance in E. aerogenes clinical isolates (Chevalier et al. 2008; Mallea et al. 1998; Masi et al. 2006). Mutation of genes implicated in the control and regulation of transporters, porins and efflux pumps also participates in development of antibiotic resistance. The marRAB operon down-regulates the outer membrane porin OmpF and upregulates the AcrAB-TolC drug efflux pump (Chollet et al. 2002; Chollet et al. 2004) and has been implicated in resistance against chloramphenicol, tetracycline and norfloxacin (George, 1996; McMurry et al. 1994).

Although no significant drug efflux has been observed in E. aerogenes strain ATCC 13048, altered transport function was induced by culturing E. aerogenes strain ATCC 13048 in the presence of chloramphenicol and imipenem (Bornet et al. 2003; Ghisalberti et al. 2005). Overexpression of MDR-associated genes in E. aerogenes strain ATCC 13048 increases its resistance to β-lactams, tetracycline, chloramphenicol and nalidixic acid (Chollet et al. 2004; Dupont et al. 2004). Resistance associated with the downregulation of porins or upregulation of efflux pumps is relatively non-specific and once E. aerogenes develops a MDR phenotype it could exclude from the cytoplasm a number of unrelated drugs, disinfectants or organic solvents.

Genomic antibiotic resistance may also arise from changes to the drug target and mutations in gyrA were reported to confer resistance to quinolone drugs. E. aerogenes strain ATCC 13048 does not contain mutation in gyrA which lead to resistance to fluoroquinolone drugs (Weigel et al. 1998). Bacteria have also been observed to create new metabolic pathways that completely bypass the primary target (Kaye et al. 2004).

Horizontal gene transfer has been recognized as one of the major mechanisms driving the evolution of micro-organisms (Ochman et al. 2000) and contributes to the development of antibiotic resistance in E. aerogenes. The acquisition of new genes or gene clusters coding fitness factors or virulence determinants, including antibiotic resistance, can provide a selective advantage or allow the micro-organism to invade new hosts and exploit new habitats. Various plasmids and integrons carry genes conferring resistance to β-lactams, chloramphenicol, imipenem, sulfonamides, trimethroprim and aminoglycosides (Appendix 3) have been isolated from E. aerogenes (Pitout et al. 1998). Interspecies transfer of plasmids allows E. aerogenes to acquire and disseminate antibiotic resistance plasmids in clinical settings (Arpin et al. 1996; Marchandin et al. 2003; Neuwirth et al. 1996; Novais et al. 2010). Plasmid-borne Class A extended-spectrum β-lactamases (ESBLs), especially those of the TEM family are the most frequently isolated and are associated with outbreaks of infection in hospital settings (Biendo et al. 2008; De Champs et al. 1991a; Mammeri et al. 2001; Narayan et al. 2009; Salso et al. 2003). IMP-1 type MBLs (metallo-β-lactamases) Class 1 integrons conferring resistance to

imipenem, aminoglycosides and β-lactams, are believed to originate from Pseudomonas aeruginosa and were found in various pseudomonads and enterobacteria including Serratia marcescens, K. pneumoniae and E. aerogenes (Shibata et al. 2003; Walsh et al. 2005). Efflux pumps implicated in MDR phenotype can also be acquired throught horinzontal gene transfer (Appendix 3).

E. aerogenes strain ATCC 13048 does not contain plasmids bearing virulence factors or antibiotic resistance to donate, but it could accept plasmids from other bacteria and disseminate them to members of the microbial community in a host or in the environment. Laboratory testing confirmed the ability of E. aerogenes strain ATCC 13048 to transfer and receive plasmids from other species (Henschke and Schmidt 1989) at rates similar to High frequency recombination (Hfr) E. coli (Curtiss 1969).

Whether it arises from genomic mutation or is gained through horizontal transfer, antibiotic resistance reduces the number of effective treatment options and is associated with more persistent infections (Arpin et al. 1996) and higher mortality (De Gheldre et al. 2001), E. aerogenes efficiency in circumventing the detrimental effects of antibiotics could pose a risk to both infected individuals and others who might become infected with resistant strains. Studies on E. aerogenes infections show that the emergence of highly antibiotic resistant and multi drug resistant E. aerogenes is associated with the use of antibiotics (Barnaud et al. 2004; Jacobson et al.1995; Lee et al. 2002; Pfaller et al. 1997). E. aerogenes strain ATCC 13048 carries many genes implicated in antibiotic resistance (Appendix 4), and therefore, has the potential to develop antibiotic resistance in the right context and could pose great risk to susceptible individuals as options for treatment of infections are potentially limited.

1.1.2.6 Pathogenic and toxigenic characteristics

E. aerogenes is an opportunistic pathogen and its emergence as a nosocomial pathogen is associated with the increased use of antibiotics and the emergence of antibiotic resistance. The ability of E. aerogenes to produce infections in both human and non-human species is not totally understood, but mechanisms that allow it to adhere to and invade a host, evade host defences and damage host tissues have been identified and could play an important role in the establishment of infection.

In vitro testing of various E. aerogenes clinical isolates showed that they have the ability to adhere and invade Hep-2 human cancer cell line (Koczura et al. 2011). Enterobacterial attachment to host cells is mediated by different types of pili, also called fimbriae. E. aerogenes is known to produce type 1 and type 3 fimbriae (Adegbola and Old 1985; Grimont and Grimont 2005; Hornick et al. 1991; Livrelli et al. 1996). Type 1 fimbriae are associated with urinary tract and respiratory infections (Hornick et al. 1991; Maayan et al. 1985; Tarkkanen et al. 1992). They can bind to mannose-containing trisaccharides on host surface glycoproteins such as those found on the epithelial cells of the urinogenital and respiratory tracts (reviewed in Clegg and Gerlach 1987; reviewed in Podschun and Ullman 1998). Type 3 fimbriae are mannose-resistant hemagglutinins that help bacteria adhere to various host cells (Hornick et al. 1992; Tarkkanen et al. 1992) but their role in pathogenesis is less clear (review in Podschun and Ullman 1998). In K. pneumonia, Type 3 fimbriae do not seem to have a direct role in establishing infection; however, they have been

identified as important for biofilm formation in the Enterobacteriaceae in general, and therefore could be an important factor in the development of infection in catheterized patients (Struve et al. 2009). Biofilm formation could increase resistance to antibiotic agents. Although E. aerogenes is not known to form biofilms on its own, it has been isolated from biofilms generated by other micro-organisms (Sharma and Anand 2002).

The presence of siderophores in E. aerogenes contributes to its ability to survive in the host tissue. The availability of iron is a limiting factor in bacterial infection. In the human body, iron is complexed with carrier molecules, such as hemoglobin, lactoferrin, and transferrin, and little free iron is accessible to micro-organisms. Under such iron limiting conditions, bacteria need high affinity system, known as siderophores, to solubilise and import iron to sustain growth (Bullen et al. 2005; Payne 1988). A number of siderophores have been found in Enterobacteriaceae. E. aerogenes is known to produce high-affinity siderpohores: a catechol (enterobactin) and a hydroxamate (aerobactin) (Koczura et al. 2011; Payne, 1988). The gene for aerobactin has also been found on a plasmid and can be horizontally transferred through conjugation (see Appendix 3).

Only one report in the scientific literature attributed exogenous toxin production to E. aerogenes. Lecithinase or α-toxin was detected in one strain of E. aerogenes isolated from food samples (Oladipo et al. 2008). Testing performed by Health Canada showed that E. aerogenes strain ATCC 13048 does not produced lecithinase.

The capsule associated with E. aerogenes contributes to its ability to evade the host immune system. Like many Gram-negative bacteria, Klebsiella species are enveloped by a capsule of repeated units of acidic polysaccharides, known as K-antigens. K-antigens protect the bacteria from phagocytosis by polymorphonuclear granulocytes and prevent serum-related killing of the bacteria by inhibition of alternative complement pathway (reviewed in Podschum and Ullman, 1998) (Simoons-Smit et al. 1986). Certain K-antigens are associated with virulence, for example, strains harbouring K:1, K:2, K:4 and K:5 were reported to be more virulent than other types in mice, and loss of K-antigens results in the reduction of virulence (Simoons-Smit et al. 1984; Simoons-Smit et al. 1986). There are 78 different K-antigens among Klebsiella species K-antigens associated with E. aerogenes or K. mobilis are mainly K:68 or K:26. Others including K:42, K:59, K:11 and the virulence associated K:4 are also encountered (Grimont and Grimont 2005).

E. aerogenes has an outer membrane that is primarily composed of lipopolysaccharide (LPS). This major membrane component is an important contributor to pathogenicity in all Gram-negative bacteria, including E. aerogenes (Bannerman et al. 2003; Bone 1993; Sanders and Sanders, 1997). LPS activates the innate immune system, which is an important response for clearance of invading micro-organisms (Freudenberg et al. 2008; Trent et al. 2006). During severe Gram-negative infections, bacterial replication and cell death lead to the release of significant quantity of LPS in the blood stream, which bind to circulating proteins and ultimately lead to the production and secretion of inflammatory cytokines (reviewed in Fenton and Golenbock 1998; Kawai and Akira 2006 McGettrick and O'Neil 2004; Peri et al. 2010; Triantafoilou and Triantafoilou 2002). Cytokines that are released could lead to sepsis, a systemic inflammatory response to infection. The cytokines

and other mediators released during an important infection act together and have important effects on major organs (kidney, liver and lungs), heart and vasculature (Akira et al. 2006). Sepsis results in septic shock when accompanied by hypotension and multiple organ dysfunctions and has a mortality rate that ranges between 20-80% and could reach 90% (Bone 1993; Parrillo et al. 1993).

Animals are also sensitive to the effect of microbial LPS. Effects of LPS have been tested in chicken, sheep, pigs, rabbits and rodents. It generally elicits an inflammatory response and release of IL-1β, IL-6 and TNF-α (Kabaroff et al. 2006; Morimoto et al. 1988; Musa et al. 2009; Webel et al. 1997). LPS is a major contributor to acute bovine mastitis caused by Gram-negative bacteria (Carroll et al. 1969; Mattila and Frost 1989).

Appendix 5 lists genes known to be implicated in the pathogenesis of E. aerogenes that were confirmed to be present in the E. aerogenes strain ATCC 13048 genome. In vitro tests conducted at Health Canada using E. aerogenes strain ATCC 13048 show that E. aerogenes strain ATCC 13048 has the ability to grow in the presence of human colonic cells (HT29) and there was some evidence of cytotoxic effects after 6 hours of exposure. No hemolytic activity was observed on sheep blood agar.

1.1.3 Effects

1.1.3.1 Environment

Vertebrates

E. aerogenes is naturally present in the gut flora of many vertebrates and acts as an opportunistic pathogen when host resistance is low (Dugalic-Vrndic et al. 2010). Since E. aerogenes is normally present in most healthy vertebrates, determining when this micro-organism is responsible for an infection can be difficult. Only cases where E. aerogenes was confirmed as the cause of infection are included in Appendix 6.

E. aerogenes infections were reported in the following vertebrates: chicken embryos, cows, armadillos, and lesser and giant anteaters (Appendix 6). Except in chicken embryos, the infections reported in vertebrates occurred without interventions that breached normal barriers to infection. Injection of a particular strain of E. aerogenes in chicken embryos was done to test the pathogenicity of that strain.

Coliforms can cause acute mastitis in the bovine mammary gland. Enterobacter species are among the most common pathogens implicated in acute mastitis (Eberhart 1984). E. aerogenes was reported to cause mastitis in experimentally infected cows (Carroll et al. 1969; Jain et al. 1971). Healthy cows recovered without treatment within two weeks of infection, whereas neutropenic cows failed to recover and the infection led to necrosis of the mammary gland, demonstrating that the immune status of cows with acute mastitis is an important factor in the outcome of the infection (Wenz et al. 2001). E. aerogenes has also been reported to cause metritis in dogs. Infected dogs were treated effectively with a combination of ciprofloxacin, gentamicin, and/or chloramphenicol. Enteric infections caused by E. aerogenes have been reported in captive armadillos and anteaters, which were

successfully treated with chloramphenicol, ampicillin, or a combination of trimethoprim and sulphamethoxazole.

E. aerogenes has been isolated from: goats, buffaloes, tilapias (Heterobranchus bidorsalis and Clarias lazera), tammar wallabies (Macropus eugenii), horses, sheep, and scromboid fish (Goda et al. 1986; Hoda et al. 1993; Okaeme, 1989; Old et al. 1998; Platt et al. 1976; Venugopal et al. 1980; Verma et al. 1982; Wakwoya 2006). E. aerogenes was not confirmed as the etiologic agent of infections in these animals; other pathogenic micro-organisms isolated along with E. aerogenes prevented accurate assessment of the cause of infection. These case reports were thus excluded from Appendix 6. Additionally, albino Mus musculus (mice) were fed wheat grain inoculated with E. aerogenes that had been isolated from diseased citrus leafminer (Phyllocnistis citrella). It was found that E. aerogenes is not pathogenic towards mice (Meca et al. 2009).

As shown in Table 1-4, E. aerogenes strain ATCC 13048 is susceptible to various antibiotics such as 3rd and 4th generation cephalosporins, carbapenems, quinolones and tetracyclines. Should E. aerogenes strain ATCC 13048 be implicated in an infection in vertebrate species; effective treatments are available.

Invertebrates

E. aerogenes was also reported to cause infection in the following invertebrates: moth larvae (Boarmia selenaria), citrus leafminer (Phyllocnistis citrella), earthworms (Hoplochaetella suctoria), beetle larvae (Trilobium castaneum) and freshwater shrimp (Appendix 6). E. aerogenes was found to be pathogenic towards P. citrella larvae but not to its predators Hippodamia convergens or Chrisoperla externa (Meca et al. 2009). The larval stages of various invertebrates appeared to be most susceptible to experimentally induced E. aerogenes infection as the effects in adults are reduced or absent. E. aerogenes infections in Boarmia selenaria, Hoplochaetella suctoria, Trilobium castaneum and freshwater shrimp resulted in the common symptoms of immobility or limited mobility, and often resulted in death. Tobacco and tomato horn worms (both are moth caterpillars) were also found to have E. aerogenes present in the gut of healthy specimens, but at higher concentrations in diseased worms. The diseased worms (Butcher et al. 1967) contained E. aerogenes along with multiple other pathogenic species making it difficult to determine if E. aerogenes was the sole cause of the infection and thus was excluded from Appendix 6.

Environment Canada[9] conducted research on the pathogenicity and toxicity of E. aerogenes strain ATCC 13048 on a soil arthropod, Folsomia candida. After a 28 day exposure to clay loam soil inoculated with 10^9 CFU of E. aerogenes strain ATCC 13048 per gram of dry soil at days 0 and 14 a significant decrease in juvenile production was observed in two independent experiments, when compared with the negative controls. As the difference in the effect between the live (73% and 25%) and killed (61% and 41%) cells was not statistically different the decrease in juvenile

[9] Tests were conducted at the Biological Assessment and Standardization Section, Soil Biotechnology Lab according to “Guidance Document for Testing the Pathogenicity and Toxicity of New Microbial Substances to Aquatic and Terrestrial Organisms (EPS 1/RM/44, March 2004)”

production may be attributable to a component of the bacterial cells that is still active in killed cells. More testing would be needed to determine the mechanism and the persistence of this adverse effect. One test showed a significant decrease in the survival of adults exposed to live (34%) and killed (28%) cells; however, this effect was not observed when the test was repeated (Environment Canada 2010).

E. aerogenes has been isolated from: juvenile American lobsters (Homarus americanus), root borers (Prionus laticollis), southern pine beetle larvae (Dendroctonus frontalis), honey bees (Apis mellifera), banded cucumber beetles (Diabrotica balteata), boll weevils (Anthonomus grandis), green lacewings (Chrysoperla rufilabris), turnip moths (Agrotis segetum), and Myrmeleon bore larvae (Benham Jr. et al. 1971; Bowser et al. 1981; Gilliam et al. 1974; Moore 1972a and 1972b; Schalk et al. 1987; Thompson et al. 1978; Woolfolk and Inglis 2004; Yoshida et al. 2001). E. aerogenes was found to be non-pathogenic to these particular invertebrate species despite its prevalence in their digestive tracts as well as other parts.

Plants

E. aerogenes was not reported to be pathogenic to plants. It has been isolated from apple tree root systems and has inhibitory effects against fungal plant pathogens such as: Pythium ultimum, Venturia inaequalis, Alternaria alternate, Monilinia fructicola, Sclerotium cepivorum, Botrytis cinerea, Botrytis allii, Pennicillium expansum, Sclerotinia sclerotiorium, Rhizoctania solani, Collitotricum lindemuthianum, and Phytophthora cactorum (Marchi and Utkhede, 1994; Utkhede and Sholberg 1986).

Tests were conducted at Environment Canada[10] to evaluate the potential of E. aerogenes strain ATCC 13048 to cause cytotoxicity and adverse effect on red clover. No significant effects in root and shoot length or dry weight were observed (Environment Canada 2010).

1.1.3.2 Human health

E. aerogenes is a commensal bacterium in the human intestinal tract that has become an important nosocomial pathogen (Chow et al. 1991; Chevalier et al. 1999; Cosgrove et al. 2002). Enterobacter species represent 6% of all isolated pathogens causing infection in hospital settings (NNIS 1996), and rank in the top four pathogens causing surgical wound infection. In intensive care patients, it is also one of the top four pathogens responsible for bloodstream and urinary tract infections (Jarvis and Martone 1992). More recently, the US National Nosocomial Infections Surveillance (NNIS) System Report indicated that Enterobacter species resistant to cephalosporins represented 27.7% of all resistant bacteria isolated from intensive care patients in the USA (Cardo et al. 2004).

[10] Tests were conducted at the Biological Assessment and Standardization Section, Soil Biotechnology Lab according to “Guidance Document for Testing the Pathogenicity and Toxicity of New Microbial Substances to Aquatic and Terrestrial Organisms (EPS 1/RM/44, March 2004)”

E. aerogenes is almost exclusively a nosocomial pathogen that generally infects immuno-compromised individuals, neonates, the elderly, individuals that have received antibiotic therapy or patients in intensive care (Bilevicius et al. 2001; Boban et al. 2011; Chow et al. 1991; Cosgrove et al. 2002; Davin-Regli et al. 1996a and b; Edwards et al. 1978; Deal et al. 2007; Loiwal et al. 1999; Nauciel et al. 1985; Narayan et al. 2009; Piagnerelli et al. 2002; Rusthoven et al. 1979; Song et al. 2010). Bacteremia has been reported in numerous cases and is often associated with catheters and other medical devices, that could lead to sepsis and ultimately to septic shock (Bilevicius et al. 2001; Blot et al. 2003; Burnichon et al. 2004; Chang et al. 2009; Chen, et al. 2008a; Chow et al. 1991;Deal et al. 2007; Désinor et al. 2004; Edwards et al. 1978; Fok et al. 1998; Galani et al. 2007; Goshi et al. 2002; Kang et al. 2004; Jalaluddin et al. 1998; Kapoor et al. 2005; Loiwal et al. 1999; Narayan et al. 2009; Nauciel et al. 1985; Rusthoven et al. 1979; Sundaram et al. 2009; Song et al. 2010). Deaths were reported in several examples of those cases (Blot et al. 2003; Chen et al. 2008a; Chow et al. 1991; Cosgrove et al. 2002; Deal et al. 2007; Song et al. 2010). Septicemia caused by E. aerogenes has been reported in two immunocompetent healthcare workers with no known underlying conditions (Jha et al. 2016). Antibiotic therapy included metronidazole, ceftriaxone and cefepime and both individuals recovered.

Enterobacter species are the third leading cause of nosocomial respiratory tract infections (Jarvis and Martone 1992). E. aerogenes respiratory tract infections usually present as pneumonia and may be associated with ventilator equipment (Jalaluddin et al. 1998; Sanders et al. 1996; Piagnerelli et al. 2002). Various other respiratory tract infections and conditions have also been reported and linked to E. aerogenes (Bornet et al. 2000; Burnichon et al. 2004; Davin-Regli et al. 1996a and b; Jalaluddin et al. 1998; Mardrus et al. 1998; Meyers et al. 1988). Community-acquired Enterobacter pneumonia has also been described (Mégarbane et al. 2004).

E. aerogenes has also been implicated in urinary tract infections, usually associated with underlying diseases or conditions and with urinary catheters or associated instrumentation (Bornet et al. 2000; Burnichon et al. 2004; Cunha et al. 2007; Davin-Regli et al. 1996ab; Goshi et al. 2002; Jalaluddin et al. 1998; Kaltenback et al. 2002; Piagnerelli et al. 2000; Piagnerelli et al. 2002; Sanders et al. 1996).

E. aerogenes has been associated with meningitis (Désinor et al. 2004; Huang et al. 2001; Khan, 2004; Mellencamp et al. 1990; Poetker et al. 2003), especially in patients who had recent histories of neurosurgery and in some cases medical devices were implicated as the source of infection (De Champs et al. 1991b; Foster et al. 2005; Hamid et al. 2007). Community-acquired infections resulting in meningitis were reported in the literature (Chang et al. 2000; Huang et al. 2001; Parodi et al. 2003). Mortality due to meningitis was reported in several instances (Mellencamp et al. 1990; Parodi et al. 2003; Foster et al. 2005).

E. aerogenes has been linked to various soft tissue infections or localised infections (Bowles and Perkins, 1999; Edouard et al. 2010; Rodrigues et al. 2012; Sanders et al. 1996; Valero et al. 1985; Walder et al. 1996). In most cases, E. aerogenes infection was acquired after a prolonged hospital stay and infection is often thought to be mediated by the use of contaminated intravenous fluids or other equipments and usually affects surgical sites.

E. aerogenes has also been implicated in an array of more rare disease manifestations including prosthetic valve endocarditis (Bouraoui et al. 2005), descending mediastinitis (Cai et al. 2006), periodontal disease (Gonçalves et al. 2007), ocular infection (Khater et al. 1997), cellulitis (Michowitz et al. 1985), gall bladder infection (Manolis, 2008), and aortitis (Rondina et al. 2006). Localised infections in bone due to E. aerogenes have also been reported including osteoaritucular infections (Syrogiannopoulos et al. 1986; Loznieweski et al. 1997), spondulodiscitis (Strady et al. 2000) and osteomyelitis (Gentry and Rodriges 1990; Lin et al. 2010; Sanders et al. 1996). E. aerogenes has also been implicated in a case of necrotizing fasciitis in a patient who has undergone fat grafting breast augmentation (Pek et al. 2015). Other organisms implicated included Enterococcus faecalis, Proteus mirabilis and Bacteroides fragilis.

E. aerogenes was identified as the causative agent in numerous hospital outbreaks, commonly arising from the contamination of medical devices or intravenous fluids. E. aerogenes has been isolated from various sources in hospital settings and is known to be well adapted in this kind of environment. E. aerogenes has been isolated from furniture and floor surface, IV solutions, shunts, and rubber piping (Arpin et al. 1996; Edwards et al. 1978; Hamid et al. 2007; Loiwal et al. 1999). In other cases, health care personnel were responsible for the transmission of the strain responsible for the infection (Piagnelli et al. 2000). The most prevalent forms of E. aerogenes infection in outbreak scenarios include bacteremia, septicemia, urinary and respiratory tract infections. These are mostly seen in immuno-compromised individuals, the elderly or neonates (Appendix 2). Outbreaks mostly occur through the clonal spread of an E. aerogenes strain carrying a specific plasmid containing antibiotic resistance genes. An outbreak of E. aerogenes carrying such plasmid led to the dissemination of antibiotic resistance to other Entrobacteriaceae (Neuwirth et al. 1996).

E. aerogenes is naturally resistant to amoxicilllin and many cephalosporins; therefore, the treatment of choice generally includes gentamicin, tobramycin, amikacin or ciprofloxacin. As discussed previously, treatment of E. aerogenes infections is hampered by resistance to antibiotics. Antibiotic sensitivity tests performed on clinical isolates show a wide variability between isolates depending on mutation or activation of chromosomal genes and horizontally acquired resistance genes. E. aerogenes is very efficient in circumventing the detrimental effects of antibiotics given during drug therapy and a severe infection can develop in immunocompromised individuals whose immune systems cannot eliminate the agent of infection. Mortality linked to E. aerogenes infections is highly associated with inadequate antibiotic therapy (Blot et al. 2003). When the infection is with ESBL-producing E. aerogenes, imipenem, meropenem, doripenem or cefepime is recommended (Goethaert et al. 2006), but increased use of these antibiotics against E. aerogenes infections has led to the development of resistance (Barnaud et al. 2004; Chollet et al. 2003; Rubio-Perez et al. 2012). As shown in Table 1-3, E. aerogenes strain ATCC 13048 is susceptible to some 3rd and 4th generation cephalosporins, carbapenems, quinolones and tetracyclines. Should E. aerogenes strain ATCC 13048 be implicated in an infection; effective treatments are available.

In in vivo tests conducted at Health Canada, BALB/c mice exposed to 10^6 colony forming units (CFU) of E. aerogenes strain ATCC 13048 by endotracheal instillation showed no changes in behavior and physical appearance. All bacteria were cleared

from the lung 24 hours after exposure. No significant increase in lung granulocytes, lung or blood cytokines, or in serum amyloid A were observed over the one week sampling period except for a transitional increase of IL1-β and TNF-α observed in the lungs after 2 hours of exposure. Secretion of cytokines is most likely a response to the presence of LPS on E. aerogenes strain ATCC 13048.

1.2 Hazard severity

1.2.1 Environment

The environmental hazard potential of E. aerogenes strain ATCC 13048 is assessed to be medium because the micro-organism is known as an opportunistic pathogen but under normal circumstances it is unlikely to be a serious hazard to healthy livestock or other organisms in the environment. Under certain conditions, it can infect some animals causing a range of symptoms that can debilitate the host and even kill it. Generally, infection causes some adverse but reversible effects, in the intermediate term, and effective treatments or mitigation measures are available. E. aerogenes can cause mastitis in cows, but affected animals recover rapidly upon treatment with veterinary antibiotics. Some invertebrates are susceptible to E. aerogenes including E. aerogenes strain ATCC 13048. Despite its prevalence and association with various environmental species and habitats, there is no evidence in the scientific literature to suggest that E. aerogenes has any adverse ecological effects at the population level for plants, vertebrates or invertebrates.

1.2.2 Human health

The human hazard potential of E. aerogenes strain ATCC 13048 is assessed to be medium because severe disease or fatality are limited to susceptible sub-populations (with compromised immune system) or were rare, localized and rapidly self-resolving in healthy humans. The vast majority of E. aerogenes-related infections occur in hospital settings and generally affect neonates, the elderly and patients in intensive care. Infections are usually treatable, but are often associated with development of antibiotic resistance. There are some reports of death related to E. aerogenes infections in humans with serious underlying conditions. The potential for transmission of infection to other humans is limited. Should E. aerogenes strain ATCC 13048 be implicated in an infection, effective treatments are currently available as it is sensitive to several clinically relevant antibiotics; however, the known capacity of E. aerogenes strain ATCC 13048 to acquire and develop antibiotic resistance elevates the assessment of hazard. Effects reported in laboratory animal models of human infection are not lethal or limited to invasive exposure routes (i.e., endotracheal instillation) or are mild and rapidly self-resolving.

Hazards related to micro-organisms used in the workplace should be classified under the Workplace Hazardous Materials Information System (WHMIS)[11].

[11] A determination of whether one or more criteria of section 64 of CEPA are met is based on an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA may not be relevant to, nor

2. Exposure assessment

2.1 Sources of exposure

This assessment considers exposure to E. aerogenes strain ATCC 13048 resulting from its addition to consumer or commercial products and its use in industrial processes in Canada.

E. aerogenes strain ATCC 13048 was nominated to the DSL based on its use in consumer and commercial products for biodegradation, biofiltration as well as water, wastewater and aquaculture treatment. It has properties that make it of commercial interest in a variety of industries.

The Government conducted a mandatory information-gathering survey under section 71 of CEPA, as published in the Canada Gazette, Part I, on October 3, 2009 (section 71 notice). The section 71 notice applied to any persons who, during the 2008 calendar year, manufactured or imported E. aerogenes strain ATCC 13048, whether alone, in a mixture or in a product. E. aerogenes strain ATCC 13048 was reported to be used in very small quantities for research and development (R&D), and teaching activities, but not for industrial, commercial or consumer uses.

A search of the public domain (internet, patent databases, and research and development literature) suggests multiple potential uses in industrial, commercial and consumer settings (Table 2-1).

Table 2-1: Potential uses identified for E. aerogenes strain ATCC 13048

Type	Description of Use	References
Bioremediation (heavy metals)	- Hexavalent chromium contaminated areas - Cadmium contaminated areas - Organophosphate pesticides (e.g., paraoxon and demeton) - Copper and Cadmium contaminated areas - Mercury contaminated areas	- Pinon-Castillo et al. 2010 - Scott, and Karanjkar, 1992 - Hadler et al. 2009 - Huang et al. 2005 - Mehta and Vaidya, 2010
Biodegradation (organic compounds)	- Mono- and polyestolides - Dimethyl phthalate and possible other phthalic acid esters - Acrylamide contaminated areas	- Erhan and Kleiman, 1997 - Perez et al. 1977 - Buranasilp and Charoenpanich, 2011
Biofuel Production	- Hydrogen - Ethanol - 2,3-Butanediol	- Han et al. 2012 - Lee et al. 2012 - Kautola et al. 1984
Biocatalysis	- N^6-hydroxylysine production - 6-aminocaproic acid production	- Parniak et al. 1979 - US 8088607
Enzyme Production	- Restriction endonuclease, Ear I - Glycerophosphodiesterase (GdpQ), a binuclear metallohydrolase - L-asparaginase, exhibits anti-tumor activity	- Polisson and Morgan, 1988 - Hadler, et al. 2009 - Geckil and Gencer, 2004

does it preclude, an assessment against the criteria specified in the Hazardous Products Regulations, which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.

Type	Description of Use	References
Wastewater treatment	Produces an acidic polysaccharide bioflocculant, named WF-1	Lu et al. 2005
Diagnostics	Antibiotic preservative efficacy testing and antibiotic resistance essay	United Kindom Patent GB2429283
Biological Control Agent	E. aerogenes is pathogenic towards P. citrella	Meca et al. 2009

2.2 Exposure characterization

The exposure characterization is based on activities reported in the notice (R&D and teaching). Measures to reduce human and environmental exposure to Risk Group 2 pathogens from their use in research and teaching laboratories are in place under the Canadian Biosafety Standard Second Edition, 2015 (CBS 2015). These include specific laboratory design, operational practices and physical requirements. For example, all material must be contained and is decontaminated prior to disposal or reuse in such a way as to prevent the release of an infectious agent, and equipment for emergency and decontamination response must be readily available and maintained for immediate and effective use. As E. aerogenes strain ATCC 13048 is a Risk Group 2 human and animal pathogen, measures to reduce human and environmental exposure from its use in research and teaching laboratories are in place under the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency's (CFIA) CBS 2015.

2.2.1 Environment

Based on the absence of consumer or commercial activity in Canada according to the section 71 notice, the overall environmental exposure estimation for E. aerogenes strain ATCC 13048 is low. Nevertheless, given the range and scale of known and potential applications of the species E. aerogenes listed in Section 2.1, there is potential for an increase in environmental exposure to products containing E. aerogenes strain ATCC 13048, and exposure scenarios arising from these products have been considered.

Should potential uses identified in Section 2.1 be realized in Canada, the most likely routes of introduction of E. aerogenes strain ATCC 13048 into the environment would be into terrestrial ecosystems from its use in bioremediation or biodegradation. Additionally, it may enter aquatic ecosystems through wastewater treatment or as runoff from applications to soil or plants or from waste effluent of commercial or industrial settings. A portion of released E. aerogenes strain ATCC 13048 is expected to remain viable and could establish communities where organic matter accumulates. In addition, large or repeated releases into the environment could temporarily elevate environmental concentrations of E. aerogenes but high numbers of cells are unlikely to be maintained in water or in soil. E. aerogenes does not form spores, and competition and microbiostasis are likely to prevent the persistence of this species above background levels (Chan et al. 1979).

In the event that commercial or industrial activities resume, the environmental exposure estimates derived from the responses to the 2009 notice to regarding E. aerogenes strain ATCC 13048 could change based on the exposure scenarios described above.

2.2.2 Human

Based on the absence of consumer or commercial activity in Canada according to the section 71 notice, the overall human exposure estimation for E. aerogenes strain ATCC 13048 is low. Nevertheless, given the range and scale of known and potential applications of the species E. aerogenes listed in Section 2.1, there is potential for an increase in human exposure to products containing E. aerogenes strain ATCC 13048 and exposure scenarios arising from these products have been considered.

Should potential uses identified in Section 2.1 be realized in Canada, Canadians would most likely be exposed to E. aerogenes strain ATCC 13048 as bystanders during the commercial or industrial application of products containing this strain or the release of waste. The extent of bystander exposure would depend on the mode of application, the volume applied, and the proximity of bystanders to the site of application, but in general is expected to be low.

Human exposure (e.g., through recreational activities) to bodies of water treated with products containing E. aerogenes strain ATCC 13048 or to which wastewater or soil runoff containing E. aerogenes strain ATCC 13048 has been released, could also result in exposure of the skin and eyes, as well as inadvertent ingestion. However dilution of products containing E. aerogenes strain ATCC 13048 is expected to significantly reduce exposure compared to direct handling of products containing E. aerogenes strain ATCC 13048.

Water treated with products containing E. aerogenes strain ATCC 13048 or soil runoff containing E. aerogenes strain ATCC 13048 could reach drinking water supply pipes intakes. Drinking water treatment processes, such as ozonation, are expected to effectively eliminate these micro-organisms and thus limit E. aerogenes strain ATCC 13048 ingestion through drinking water (Dosti et al. 2005; Sharma and Anand 2002a).

No consumer products containing E. aerogenes strain ATCC 13048 were identified in the public domain. However, should new application of E. aerogenes strain ATCC 13048 be developed as consumer or household products, direct human exposure could be higher, and handling and application of such products are expected to result in exposure of the skin and the eyes, inhalation of aerosolized droplets or dusts containing E. aerogenes strain ATCC 13048 or inadvertent ingestion. Exposure to bystanders is also expected to be increased.

In the event that commercial or industrial activities resume, the human exposure to E. aerogenes strain ATCC 13048 are expected to change based on the exposure scenarios described above.

3. Risk characterization

In this assessment, risk is characterized according to a paradigm whereby a hazard and exposure to that hazard are both required for there to be a risk. The risk assessment conclusion is based on the hazard, and on what is known about exposure from current uses.

Hazard has been estimated for E. aerogenes strain ATCC 13048 to be medium for the environment and for human health. Environmental and human exposure to E. aerogenes strain ATCC 13048 is not currently expected (low exposure), so the risk associated with current uses is estimated to be low for both the environment and human health.

The determination of risk from current uses is followed by consideration of the estimated hazard in relation to foreseeable future exposures (from new uses).

Risks to the environment from foreseeable future uses:

E. aerogenes is a known cause of acute bovine mastitis. Although the majority of cases are self-limiting and resolve without therapy, some are severe enough to cause death. Research on mastitis links a high incidence of infection with a high concentration of the pathogen in the immediate environment. Cows could be exposed to elevated concentrations of E. aerogenes strain ATCC 13048 from its use in bioremediation or biodegradation application in contaminated sites adjacent to farms or pastures. This is expected to be a rare occurrence, so the overall risk to cows from this use is expected to be low. Cows could also be exposed to elevated concentrations of E. aerogenes strain ATCC 13048 through its use in water or wastewater treatment should products containing the micro-organism be applied to cattle dugouts or irrigation ponds or should treated wastewater or biosolids be applied to agricultural land. The overall risk from these uses to the Canadian dairy herd is nevertheless expected to be low. Exposure via ingestion of E. aerogenes strain ATCC 13048 through drinking water is not a route of concern for mastitis. Direct contact of cows to soil treated or contaminated with E. aerogenes strain ATCC 13048 could lead to mastitis; however, the risk associated with this exposure remains low because livestock already live in the farm environment containing high concentrations of Enterobacteriaceae, and because the majority of mastitis infections are self-resolving or treatable with effective antibiotic drugs against the DSL strain.

E. aerogenes was reported to be harmful to certain invertebrates, including larvae of certain insects, which is the basis for its potential use as a biocontrol agent. Inadvertent exposure of terrestrial invertebrates to E. aerogenes strain ATCC 13048 could occur through its application to contaminated sites for the bioremediation or biodegradation of toxic heavy metals or organic compounds. The overall risk to susceptible terrestrial invertebrates is expected to be low because life of these organisms at such sites is already jeopardized by the presence of toxic contaminants, and because such sites represent a very small proportion of the Canadian land mass. Terrestrial or aquatic invertebrates may also be exposed to E. aerogenes strain ATCC 13048 through its use in water and wastewater treatment or as runoff from applications to soil or plants or from waste effluent of commercial or industrial settings. However, the dilution factor of products containing E. aerogenes strain ATCC 13048 is expected to be significant, so the concentrations required to see adverse effects are not anticipated be reached.

Owing to the above considerations, the use of E. aerogenes strain ATCC 13048 in bioremediation or biodegradation or water and wastewater treatments is unlikely to have a long term impact on invertebrate populations and population trends over an

entire ecosystem or an ecozone. Based on the considerations outlined above, the risk to the environment from foreseeable future uses is expected to be low.

Risks to human health from foreseeable future uses:

In humans, E. aerogenes is mostly reported as a nosocomial pathogen that leads to a wide array of infections, including wound infections, meningitis, respiratory and urinary tract infections, bacteremia, septicemia and septic shock. The risk of E. aerogenes infection is associated with antibiotic use, the presence of indwelling medical devices, long hospital stays and immune deficiency of the individual. E. aerogenes has been isolated from various sources in hospital settings and is known to adapt well in this kind of environment. E. aerogenes is also well known for its ability to develop antibiotic resistance, and although, strain ATCC 13048 is currently susceptible to many antibiotics, it has the ability to adapt quickly to selective pressure applied through the use of antibiotic drugs and acquire resistance genes from other organisms. E. aerogenes strain ATCC 13048 carries many genes implicated in resistance, and therefore, has the potential to develop antibiotic resistance in the right context and could pose great risk to susceptible individuals. While it is also possible that E. aerogenes strain ATCC 13048 may acquire mobile genetic elements from its environment, the probability of such an occurrence is no higher than for other naturally occurring or commensal Enterobacteriaceae. The DSL strain does not contain plasmids bearing virulence factors or antibiotic resistance genes, so it cannot be implicated in the conjugal transfer of virulence factors to other bacteria in the environment.

The use of products containing E. aerogenes strain ATCC 13048 in hospitals or tertiary care centres could pose health risks to susceptible populations, including the elderly and neonates.

4. Conclusion

Based on the information presented in this screening assessment, it is concluded that E. aerogenes strain ATCC 13048 is not entering the environment in a quantity or concentration or under conditions that:

- have or may have an immediate or long-term harmful effect on the environment or its biological diversity;
- constitute or may constitute a danger to the environment on which life depends; or
- constitute or may constitute a danger in Canada to human life or health.

Therefore, it is concluded that E. aerogenes strain ATCC 13048 does not meet the criteria as set out in section 64 of the CEPA.

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Appendices

Appendix A: Growth of E. aerogenes strain ATCC 13048 in various media

Table A-1: Growth of of E. aerogenes strain ATCC 13048 in liquid media at various temperatures

Medium	28°C	32°C	37°C	42°C
Trypticase Soy Broth	+	+	+	+
Sheep Serum	~	~	~	~
Fetal Bovine Serum	+	+	+	(+)
Dulbecco's Modified Eagles Medium	+	+	–	–

– indicates no growth, + indicates growth, ~ indicates low level growth, (+) indicates delayed growth (after 15h)
Data generated by Health Canada's Environmental Health Science and Research Bureau. Growth of E. aerogenes strain ATCC 13048 in broth culture, as measured by increase in absorbance at 500 nm, in four different growth media and over a range of temperatures: Concentration of bacteria at time zero was 1×10^6 CFU/mL. Measurements were taken every 15 minutes over a 24-hour period with a multi-well spectrophotometer.

Table A-2: Growth characteristics of E. aerogenes strain ATCC 13048 on solid media at various temperatures

Medium	28°C	37°C
Citrate[a]	N/A	+
Lysine Iron[b]	+	+
Growth on MacConkey Agar[c]	+	+
Lactose fermentation on MacConkey Agar[c]	+	+
Mannitol Salt Agar[d]	-	-
Growth on Starch agar[e]	N/A	+
Starch Hydrolysis[e]	N/A	+
Triple Sugar Iron - with phenol red[f]	+	+
Growth on Urea agar[g]	+	+
Hydrolysis of Urea[g]	-	-
Catalase activity on TBS[h]	+	N/A
Growth on Sheep blood agar[i]	+	+
Hemolysis of Sheep blood[i]	-	-

+ indicated positive for growth or test; - indicated negative for growth or test; N/A indication data not available
Data generated by Health Canada's Environmental Health Science and Research Bureau

a Citrate utilization test, ability to use citrate as the sole carbon source

b Simultaneous detection of lysine decarboxylase and formation of hydrogen sulfide

c Detection of coliform organisms; tests for ability of organism to ferment lactose

d Isolation and differentiation of Staphylococci

e Differential medium that tests the ability of an organism to produce extracellular enzymes that hydrolyze starch

f Gram-negative enteric bacilli based on glucose, lactose, and sucrose fermentation and hydrogen sulfide production

g Screening of enteric pathogens from stool specimens - Urea metabolism

h Catalase enzyme assay measures by enzymatic detoxification of hydrogen peroxide

i Hemolysis of sheep blood. Bacteria (5000 CFU, 20 µl) were spotted onto the blood-agar and incubated for 24h

Appendix B: Outbreaks in humans

Table B-1: E. aerogenes outbreaks in humans

Country	Location	Year(s)	Cases	Synopsis	Reference
USA	Children's Memorial Hospital	1976	7	Septicemia in seven infants, outbreak due to infusion intravenous fluid	Edwards et al. 1978
France	Clermont-Ferrand Hospital	Jan. 1988-Aug. 1989	219	Spread of three ESBLs (CTX-1, CAZ-5, CAZ-6) in E. aerogenes and K. pneumoniae in hospital setting	de Champs et al. 1991a
France	Hopital Salvator	Sept. – Dec. 1993	10	Patients colonized with or infected by one strain of E. aerogenes without antibiotic resistance	Davin-Regli et al. 1996b
France	Hopital Pellegrin	Jan. 1993 - Oct. 1993	73	109 E. aerogenes isolated from urine, respiratory tract, pus of ill patients; outbreak due to a cephalosporinase-derepressed strain that later acquired antibiotic resistance plasmids	Arpin et al. 1996
France	Hopital Universitaire du Bocage	1993 - 1994	10	10 isolates of TEM-24-producing E. aerogenes (blood, urine, stool) and detection of TEM-24 in Escherichia coli and Citrobacter freundii after the outbreak suggests dissemination of TEM-24	Neuwirth et al. 1996
Belgium	Erasme Hospital	1994 - 1995	34	Single strain of multi-drug resistant E. aerogenes in ICUs causing pneumonia, urinary tract infection and bacteremia	De Gheldre et al. 1997
Belgium	Saint-Pierre University Hospital	1995	33	45 isolates of E. aerogenes from 22 infected patients and 11 colonized, outbreak due totwo clonal groups	Jalaluddin et al. 1998
Austria	University Hospital of Innsbruck	May - June 1995	7	E. aerogenes isolates (blood, stool, tracheal secretion, urine , nasal swab) undistinguishable, four from confirmed outbreak	Allerberger et al. 1996
India	University College of Medical Sciences and Guru Tegh Bahadur Hospital	1995 - 1996	13	E. aerogenes causing septicemia in a neonatal ICU; source of outbreak rubber pipe of a suction machine in ICU, six deaths	Loiwal et al. 1999
France	Brest Hospital	Jan. 1996 - Aug. 1997	26	Isolation of E. aerogenes in 26 infants, 11 of which infected and 15 colonized and recovered from breast milk	Burnichon et al. 2004

Country	Location	Year(s)	Cases	Synopsis	Reference
France	University-affiliated hospital center of Amiens	Oct. 1996-Aug. 1999	165	Total of 743 E. aerogenes isolates and 165 of them TEM-24 producing clone	Mammeri, H. et. al. 2001
Belgium	Erasme Hospital	1998	12	12 E. aerogenes isolates, seven in patients with urinary tract infection and other only colonized (rectal swab); one clone was responsible for majority of cases	Piagnerelli et al. 2000
Spain	Hospital Clinico San Carlos	Dec. 2000-Jan. 2001	10	10 TEM-24 ESBL-producing E. aerogenes isolates of same clonal origin isolated from urine, blood, scab or wound	Salso et al. 2003
Fiji	Colonial War Memorial Hospital	May 2007	10	10 ESBL-producing E. aerogenes isolates in neonatal ICU causing septicemia in neonates by a single strain, three infants died	Narayan et al. 2009

Appendix C: E. aerogenes mobile genetic elements

Table C-1: Plasmids found in strains of E. aerogenes and associated traits

Name	Group	Strain	Fitness or resistance traits	References
pRYC103T24	Inc A/C_2	N/A	Multi-drug resistance against chloramphenicol, sulfonamides, trimethoprim, β-lactams, aminoglycosides and resistance to mercury	Machado et al. 2007; Novais et al. 2010
pIP833	N/A	BM2688	Resistance to amikacin, kanamycin, tobramycin, netilmicin, sulfonamides, quaternary compounds, ticarcillin, cetyltrimethylammonium bromide	Ploy et al. 1998
pCFF04	7/M	KpCF104	• Resistance to all β-lactams, including oxyiminocephems, except cephamycins and imipenem. • Additional resistance against aminoglycosides (amikacin, kanamycin, netilmicin, tobramycin), sulphonamides and tetracyclines	de Champs et al. 1991a
pCFF74	7/M	KpCF1104	• Resistance to all β-lactams except cephamycins and imipenem, with a high level of resistance to ceftazidime and aztreonam. • Also attributes resistance to aminoglycosides (amikacin, kanamycin, netilmicin, tobramycin), sulphonamides and tetracyclines	de Champs et al. 1991a
pCFF041	6/C	KpCF1041	Resistance to all β-lactams, including oxyiminocephems (except cephamycins and imipenem), aminoglycosides (amikacin, kanamycin, netilmicin, tobramycin), sulphonamides, trimethoprim and chloramphenicol	de Champs et al. 1991a
pSMN1	N/A	Strain 62-1	Secretion of iron chelating siderophore aerobactin	McDougall and Neilands 1984; Waters and Crosa 1988
pOLA52	IncX1	DSM30053	• Efflux pump system giving resistance to olaquindox, chloramphenicol, and ethidium bromide • Resistance to ampicillin • Type 3 fimbriae	Burmolle et al. 2008; Norman et al. 2008
pEA1509_A	N/A	EA1509E	• Resistance to β- lactams, aminoglycosids, sulfamids • Resistance to mercury • ABC transporter	Diene et al. 2012
N/A	IncF	YS10 & YS11	Fluoroquinolone efflux pump	Kang et al. 2009; Park et al. 2009

N/A indicates specific strain information not available

Table C-2: Integrons found in E. aerogenes and associated traits

Group	Strain	Resistance traits	References
Class 1	MPU 5E MPU 29E	Resistance against streptomycin, spectinomycin, netilmicin, chloramphenicol, ticarillin, piperacillin, piperacillin + tazobactam, co-trimoxazole, sulfamethoxazole	Koczura et al. 2011
Class 1	N/A	• IMP-1 type MBLs (metallo-β-lactamases) • Resistance to aminoglycosides, broad spectrum β-lactams, imipenem	Shibata et al. 2003; Walsh et al. 2005
Class 1	N/A	• ESLB • Resistance to sulphonamides (sul1 and sul2 gene) • Resistance to trimethoprim (dfrA2d)	Frank et al. 2007

N/A indicates specific strain information not available

Appendix D: Genes implicated in antibiotic resistance present in the E. aerogenes strain ATCC 13048 genome (CP002824)

Table D-1: Genes implicated in neutralizing the antibiotic

Gene symbol	Characteristic/gene function
ampC	β-lactamase, degradation of antimicrobial agent (penicillins and cephalosporins)
ampD	N-acetylmuramoyl-L-alanine amidase, regulation of ampC expression
None	Chloramphenicol acetyltransferase (EC 2.3.1.28), deactivation of chloramphenicol

Table D-2: Genes implicated in modifying transport of the antibiotic

Gene symbol	Characteristic/gene function
marA	Transciptional activator marB
marB	Multiple antibiotic resistance pump, efflux of multiple antimicrobial agent
marR	Transcriptionsal repressor of marB
acrA	Transciptional activator of acrB
acrB	Multidrug efflux pump, efflux of multiple antimicrobial agent
acrR	Transcription repressor of multidrug efflux pump acrB
tolC	Type 1 secretion outer membrane protein, efflux of multiple antimicrobial agent
None	Outer membrane protein, OMP85 family, porin

Appendix E: Virulence factors present in the E. aerogenes strain ATCC 13048 genome (CP002824)

Table E-1: Virulence factor genes in E. aerogenes

Virulence factor	Genes
Type 1 Fimbriae	• Anchoring protein FimD • Regulatory protein FimB and FimE • Major subunit FimA • protein FimI
Siderophore	• Enterobactin synthetase, EntE and EntF • Aerobactin siderophore receptor, IutA • Iron-hydromaxate transporter, FhuABC
LPS	Various genes implicated in LPS assembly and periplasmic transport

Appendix F: E. aerogenes pathogenicity in non-human species

Details of infection mentioned in Section 1.1.3.1. The following two tables provide information specific to invertebrates and vertebrates, respectively.

Table F-1: Reports of adverse effects in vertebrates

Host organism	Details of infection	Outcome/other information	Reference
Chicken embryo	Inocula were prepared from cultures isolated from honey bee intestine. Bacterial suspensions were inoculated into the allantoic cavity of chicken embryos. Embryos inoculated with E. aerogenes died on the second day after inoculation. The allantochorion was necrotic.	E. aerogenes is part of the normal microflora of A. mellifera intestine E. aerogenes is pathogenic to chicken embryos.	Dugalic-vrndic et al. 2010
Female Dogs	E. aerogenes was isolated from vaginal swabs of bitches with metritis.	E. aerogenes is considered in general as an opportunistic pathogen in dogs	Shambulingappa and Manegar 2010
Cows (Holstein-Friesian)	E. aerogenes was inoculated into cow teats to experimentally induce infection. Inoculated healthy cows developed acute mastitis. Inoculated neutropenic cows developed only slight swelling initially which progressed to an extreme inflammatory response.	Healthy cows with acute mastitis recovered within 2 weeks. Neutropenic cows failed to recover. Infection led to necrosis and irreversible damage to the mammary gland.	Jain et al. 1971
Cows (Holstein-Friesian)	E. aerogenes was isolated from dairy herd in which mastitis due to Streptococcus agalactiae had been eliminated. Inocula were prepared by streaking broth cultures on blood agar plates and were reinjected into cows. All injected cows developed mastits.	One cow died of mastitis.	Carroll et al. 1969
Cows	Case reports on four different herds presenting with mastitis. E. aerogenes was found to	The incidence of E. aerogenes infections was found to be directly proportional to the number of organisms on the teat skin.	Jasper et al. 1975

Host organism	Details of infection	Outcome/other information	Reference
	be responsible for 10% of coliform organisms recovered from mastitis cases in 8 herds.		
Armadillos (Dasypus novemcinctus, Euphractus sexcinctus, Cabassous sp., Tolypeutis sp., Priondontes maximus)	Captive armadillos in Sao Paulo Zoo, Brazil. E. aerogenes was isolated from faecal samples of specimens with enteric infections.	No deaths occurred due to infection. Armadillos were treated with chloramphenicol, ampicillin, or trimethoprim + sulphamethoxazole until no signs of infection or negative laboratory results.	Diniz et al. 1997
Lesser and Giant Anteaters (Tamandua tetradactyla, and Myrmecophaga tridactyla)	Captive anteaters in Sao Paulo Zoo, Brazil E. aerogenes was isolated from faecal samples of specimens with enteritis.	Infected specimens were treated with trimethoprim + sulphamethoxazole or chloramphenicol until laboratory tests were negative for E. aerogenes and no clinical signs of infection were present. E. aerogenes is an enteric pathogen of Tamandua tetradactyla and Myrmecophaga tridactyla and can cause septicemia. No deaths due to infection were reported in this study.	Diniz et al. 1995
Chinchilla (Chinchilla lanigera)	Fatal outbreak in a major breeding colony. Inadequate nutrient supply (especially fiber), poor ventilation system and high level of relative humidity was suspected to have brought on outbreak caused by opportunistic pathogens E. aerogenes and Proteus mirabilis. Signs of infection included lethargy, anorexia, conjunctivitis, coughing, heavy diarrhea alternated with constipation, and pyrexia.	Disease usually lasted one week but death could occur 12 to 48 hours after first signs of infection. Treatment was initiated with 300mg/day/animal of chloramphenicol and oral enrofloxacin given at 200mg/L of drinking water for 21 days, resulted in limited clinical improvement. Both E. aerogenes and P. mirabilis were isolated from intestinal content and lungs of carcasses and are considered opportunistic pathogens towards Chinchilla lanigera.	Bautista et al. 2007

Table F-2: Reports of adverse effects in invertebrates

Host organism	Details of infection	Outcome/other information	Reference
Giant Looper Moth Larvae (Boarmia selenaria)	Epizootic infection caused by E. aerogenes and P. mirabilis in laboratory mass breeding. Bacterial isolates collected from homogenized infected larvae. Laboratory induced infections were performed by smearing isolated bacterial suspension onto avocado leaves fed to specimens. Specimens exhibited loss of appetite, slow movement, and intact but flaccid body wall.	Mortality in medium and large larvae of both naturally infected group and laboratory infected group. E. aerogenes alone had lower mortality rate than its combination with Proteus mirabilis.	Wysoki and Raccah 1980
Citrus leaf miner (Phyllocnistis citrella)	Diseased P. citrella larvae collected from Lime tree citrus crops (Citrus aurantifolia) E. aerogenes, Serratia sp., and Pseudomonas sp. isolated from the carcass.	Mortality occurred in diseased P. citrella. In E. aerogenes laboratory induced infections, P. citrella had an average of 71.9% after 48hours. E. aerogenes caused no adverse effects to the host plant C. aurantiflora or representative mammal M. musculus.	Meca et al. 2009
Earthworm (Hoplochaetella suctoria)	Earthworms were collected from various naturally occurring soils in India over the course of three years. Laboratory induced infection through smearing of specific microbial cultures (isolated from diseased worms) over earthworm epidermis.	E. aerogenes was shown to be the sole pathogenic species causing disease in earthworms with nearly all resulting in death. 6 days after infection, spots spread across entire body with worms becoming inactive and death ensues. Histological sectioning of diseased worms showed patchy necrosis of the epidermal region. Diseased worms were more frequent in kitchen drainage and cow dung with decaying leaves. Healthy worms became diseased when placed in pots previously inhabited by diseased worms.	Rao et al. 1983

Host organism	Details of infection	Outcome/other information	Reference
Red Flour Beetle larvae (Trilobium castaneum)	E. aerogenes cultures were isolated from gut of T. castaneum. Diet dilution technique was used to experimentally infect T. castaneum. Infected larvae were slightly-immobile before death.	Examination of hemocoel and gut of dead larvae revealed large numbers of E. aerogenes. Larvae infected with E. aerogenes had a mortality of 94.3% while controls had 5.5% mortality due to contamination E. aerogenes is highly pathogenic to larval stage but not to post-larval stages.	Kumari and Neglund 1985
Freshwater Shrimp (species not specified)	Naturally infected freshwater shrimp reared in artificial tanks. Experimental infections were also induced using bacterial isolates from the naturally infected specimens.	Infected shrimp showed cuticular lesions.	Aly and El-Attar 2001