The Isolation of *Aeromonas* Species and Other Common Enteric Bacterial Pathogens from Patients with Gastroenteritis in an Australian Population

Christopher Yuwono 1, Michael C. Wehrhahn 2,*, Fang Liu 1, Stephen M. Riordan 3 and Li Zhang 1,*

1 School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW 2052, Australia; c.yuwono@student.unsw.edu.au (C.Y.); fang.liu@unsw.edu.au (F.L.)
2 Douglass Hanly Moir Pathology, 14 Giffnock Ave, Macquarie Park, NSW 2113, Australia
3 Gastrointestinal and Liver Unit, Prince of Wales Hospital, University of New South Wales, Sydney, NSW 2031, Australia; Stephen.Riordan@sesiachs.health.nsw.gov.au
* Correspondence: MWehrhahn@dhm.com.au (M.C.W.); L.Zhang@unsw.edu.au (L.Z.)

Abstract: *Aeromonas* species are emerging human enteric pathogens. However, systematic analysis of *Aeromonas* species infection in human gastroenteritis in comparison with other enteric bacterial pathogens in the Australian population is lacking. Here we analysed the isolation of *Aeromonas* species and other bacterial pathogens in five consecutive years (2015–2019) from 375,842 stool samples of patients with gastroenteritis in a large Australian diagnostic laboratory and identified a subset (48 isolates) of *Aeromonas* isolates to species level, using multilocus phylogenetic analysis. *Aeromonas* species were the third most common bacterial pathogens, following *Campylobacter* and *Salmonella* species. *Aeromonas* infection rate was significantly correlated with increasing age (p < 0.001). *Aeromonas* species were more often isolated in warm seasons and in males than females (p < 0.001). Five *Aeromonas* species were identified. Most of the infections were from three species, namely *Aeromonas veronii* (52%), *Aeromonas caviae* (27%) and *Aeromonas hydrophila* (12.5%). The majority of patients with *Aeromonas* species infection did not have a documented overseas travel history. The findings from this study support the importance of *Aeromonas* species in human gastroenteritis and suggest that the sources of *Aeromonas* infection in Australian patients should be further investigated.

Keywords: *Aeromonas*; *Aeromonas veronii*; *Campylobacter*; *Salmonella*; gastroenteritis

1. Introduction

Members of the *Aeromonas* genus, which currently contains 36 species, are Gram-negative rod-shaped motile or non-motile facultative anaerobes [1]. *Aeromonas* species cause diseases in both humans and animals, such as fish and reptiles. A majority of *Aeromonas* infections in humans are caused by five *Aeromonas* species, namely *Aeromonas caviae*, *Aeromonas veronii*, *Aeromonas dhakensis*, *Aeromonas hydrophila* and *Aeromonas media* [1].

*Aeromonas* species cause a range of human diseases. The extra-intestinal diseases caused by *Aeromonas* species in humans most commonly consist of wound infection and bacteraemia [1–7]. *Aeromonas* species are also emerging human enteric pathogens, causing various gastrointestinal disorders, such as gastroenteritis [7–13], traveller’s diarrhoea [12] and biliary tract infections [14–16]. *Aeromonas veronii* was also found to be more prevalent in patients with inflammatory bowel disease [17].

A variety of virulence factors in *Aeromonas* species, such as flagella, pili, capsules, enterotoxins, extracellular degradative enzymes and haemolysins, have been reported [18–21]. A Shiga-like toxin was also detected in *Aeromonas* species isolated from patients with gastroenteritis [22,23].

Despite the increasing importance of *Aeromonas* species in causing human gastroenteritis, systematic analysis of their infection in human gastroenteritis in comparison with...
other human enteric bacterial pathogens in Australian population is lacking. In this study, we analysed the isolation of *Aeromonas* species and other enteric bacterial pathogens in five consecutive years, from 2015 to 2019, using data from a large private Australian diagnostic laboratory serving predominantly community-based patients. A subset of *Aeromonas* isolates was also identified at the species level.

2. Materials and Methods

2.1. Clinical Data of Enteric Bacterial Pathogen Isolation

The data analysed in this retrospective study were provided by the diagnostic laboratory Douglass Hanly Moir Pathology. The laboratory is based in Sydney, Australia, receiving specimens from the state of New South Wales. The data analysed in this study were isolations of bacterial pathogens from stool samples of patients with gastroenteritis in this laboratory during 2015–2019. The identification of enteric bacterial pathogens was based on the standard protocols in this laboratory. The analysis focussed on *Aeromonas* species, with an additional five enteric pathogens also analysed for comparison. The isolation of *Aeromonas* species was conducted by following the procedures for isolation of enteric bacterial pathogens at the Douglass Hanly Moir Pathology. Briefly, the presumptive *Aeromonas* isolates were collected from either xylose lysine deoxycholate (XLD) agar plates (pale yellow or yellow pink flat colonies), thiosulfate citrate bile-salts sucrose (TCBS) agar plates (yellow or green flat colonies) or horse blood agar (HBA) plates containing an ampicillin AMP25 disc (Oxoid™, Scoresby, VIC, Australia). The presumptive *Aeromonas* isolates collected from XLD and HBA plates were subjected to an oxidase test. The oxidase-positive isolates from XLD and HBA plates, as well as isolates from TCBS plates, were then further subjected to matrix assisted laser desorption ionization/time of flight mass spectrometry (MALDI/TOF-MS) (Vitek MS, BioMerieux, North Ryde, NSW, Australia) for *Aeromonas* identity confirmation. Confirmed isolates were reported as “*Aeromonas* species” given the potential for misidentification of *Aeromonas* beyond the genus level by MALDI/TOF-MS. Other enteric bacterial pathogens were identified either to the genus or species level.

2.2. Statistical Analyses

A logistic regression analysis was performed by using a binomial generalised linear model to assess the associations between patient age and gender with the isolation rates of different bacterial pathogens. The odds ratio, the 95% confidence interval and the p-value were generated by the model. For this part of the analysis, 287 individuals whose age and gender identities were not disclosed in the information provided to the diagnostic laboratory were excluded. Spearman’s rank correlation coefficient was used to examine the correlations between the isolation rates of different bacterial pathogens with the local monthly mean maximum temperatures from January 2015 to December 2019 that were obtained from the Australian Government Bureau of Meteorology website. The statistical analyses were performed by using R software (v. 4.0.4), along with RStudio (v. 1.4.1106), and p < 0.05 indicates a statistical significance.

2.3. Identification of Aeromonas Species

The identification of *Aeromonas* species was conducted for 48 randomly selected *Aeromonas* isolates obtained during 2019, using multilocus phylogenetic analysis (MLPA) of seven housekeeping genes, namely *gyr*B, *rpo*D, *gyr*A, *rec*A, *dnaJ*, *dnaX* and *atp*D, as previously described [24]. We sequenced the genomes of these 48 *Aeromonas* isolates for a different study, the sequences of seven genes were extracted from the sequenced bacterial genomes and used in this study for *Aeromonas* species identification. The sequences of these seven housekeeping genes of known 32 *Aeromonas* species (three strains from each species were used, if available) were obtained from the NCBI genome database. The genomes of the remaining four species, namely *Aeromonas enterica*, *Aeromonas crassostreae*, *Aeromonas aquatilis* and *Aeromonas intestinalis*, were not available on NCBI, and the reported identification of these four species was only based on the partial sequences of
six housekeeping genes; therefore, they were excluded for analysis. Phylogenetic trees were generated by the neighbour-joining and maximum likelihood methods, as previously described, using the MEGA X program [25–27]. The sequences of the seven housekeeping genes of the 48 Aeromonas species analysed in this study were submitted to GenBank under accession numbers MW837843–MW837890, MW837891–MW837938, MW837939–MW837986, MW837987–MW838034, MW838035–MW838082, MW838083–MW838130 and MW838131–MW838178.

2.4. Ethics Approval

This is a retrospective study analysing data obtained from a diagnostic laboratory that did not involve patient consent. This project has been approved by the University of New South Wales HREAP Executive (HC200755).

3. Results

3.1. Aeromonas Species Were the Third Most Common Bacterial Pathogens Isolated from Stool Samples of Patients with Gastroenteritis Following Campylobacter and Salmonella Species

A total of 375,842 stool samples processed for detection of enteric bacterial pathogens, using bacterial cultivation, at the Douglass Hanly Moir Pathology laboratory, during 2015–2019, were analysed. The isolated bacterial enteric pathogens during 2015–2019 are shown in Table 1; these pathogens were reported in this diagnostic laboratory at either genus or species level. Aeromonas species were the third most common enteric bacterial pathogens, with the isolation rate (positive isolations in 10,000 samples) being 56.73. Campylobacter species were the most common enteric bacterial pathogens, and the isolation rate was 308.56. Salmonella species were the second most common enteric bacterial pathogens, with the isolation rate being 138.09. The isolation rates of Shigella species, Plesiomonas shigelloides and Vibrio species were 6.84, 6.07 and 2.89, respectively. Various other enteric bacterial pathogens were also isolated, but with low isolation rates; their data were therefore not further analysed (Table 1).

3.2. Aeromonas Species Were the Sole Enteric Bacterial Pathogens in Most of the Positive Cases

Aeromonas species were the sole enteric bacterial pathogens in 82% (1755/2132) of stool samples that were positive for Aeromonas species isolation (Table 2). In the remaining 18% (377/2132) of samples, Aeromonas species were co-isolated with other human enteric pathogens (Table 2).

3.3. Travel History of Patients with Positive Aeromonas Species Isolation

Of the patients who were positive for Aeromonas species isolation in the five years between 2015 and 2019, 4.08% of them had “travel” or “overseas” listed as part of the clinical history supplied. These were 3.97% and 7.03% for patients who were positive for isolation of Campylobacter and Salmonella species, respectively. For patients who were positive for P. shigelloides, Shigella and Vibrio species, 23.68%, 20.54% and 21.3% had travel history, respectively.
Table 1. Isolation of bacterial pathogens from faecal samples of patients with gastroenteritis in years 2015–2019 *.

| Pathogen                  | 2015  | 2016  | 2017  | 2018  | 2019  | Five-Year Total | Isolation Rate |
|---------------------------|-------|-------|-------|-------|-------|----------------|----------------|
| Campylobacter species     | 2564  | 2282  | 1973  | 2201  | 2577  | 11597          | 308.56         |
| Salmonella species        | 1155  | 1290  | 982   | 849   | 914   | 5190           | 138.09         |
| Salmonella Paratyphi A    | 1     | 3     | 0     | 0     | 0     | 4              | 0.11           |
| Salmonella Typhi          | 1     | 1     | 1     | 2     | 0     | 5              | 0.13           |
| Aeromonas species         | 357   | 381   | 420   | 472   | 502   | 2132           | 56.73          |
| Yersinia enterocolitica   | 4     | 5     | 3     | 6     | 11    | 29             | 0.77           |
| Yersinia enterocolitica group | 9  | 15   | 4     | 7     | 4     | 39             | 1.04           |
| Yersinia frederiksenii   | 1     | 1     | 1     | 0     | 0     | 3              | 0.08           |
| Edwardsiella tarda       | 2     | 5     | 4     | 0     | 5     | 16             | 0.43           |
| Comamonas testosteroni   | 0     | 0     | 0     | 1     | 1     | 2              | 0.05           |
| Arcobacter species       | 0     | 0     | 0     | 1     | 1     | 1              | 0.03           |
| Shewanella algae         | 0     | 1     | 0     | 0     | 0     | 1              | 0.03           |

* Numbers in the table are positive isolations.

Table 2. Isolation of Aeromonas species alone or co-isolation with other enteric bacterial pathogens in years 2015–2019 *.

| Pathogen                  | 2015  | 2016  | 2017  | 2018  | 2019  | Five-Year Total |
|---------------------------|-------|-------|-------|-------|-------|----------------|
| Aeromonas                 | 277   | 307   | 354   | 390   | 427   | 1755          |
| Aeromonas, Campylobacter  | 49    | 45    | 48    | 68    | 59    | 269           |
| Aeromonas, Salmonella     | 24    | 22    | 11    | 6     | 12    | 75            |
| Aeromonas, Plesiomonas    | 3     | 2     | 3     | 5     | 2     | 15            |
| Aeromonas, Shigella       | 1     | 1     | 1     | 0     | 0     | 3             |
| Aeromonas, Vibrio         | 3     | 2     | 3     | 1     | 0     | 9             |
| Aeromonas, Yersinia       | 0     | 2     | 0     | 2     | 2     | 6             |

3.4. Isolations of Aeromonas Species and Additional Five Enteric Bacterial Pathogens in Patients of Different Age Groups and Gender

The Aeromonas species isolate rates during the five years between 2015 and 2019 are shown in Figure 1A. The Aeromonas species isolation rate was low in young children of 0–4 years old (21.82/10,000). It then gradually increased as the children got older, and it reached a peak in the age group of 20–29 years old, with the isolation rate being 59.85. Aeromonas species isolation rates in individuals of the 30–39-years-old and 40–59-years-old groups then decreased, with the isolation rates in these two age groups being 49.27 and 54.51, respectively. Interestingly, the Aeromonas species isolation started to increase again in the age group of 50–59 years old (isolation rate being 63.42), which continued to increase...
and reached a second peak in individuals over 80 years old (isolation rate being 97.73). There was a significant association between Aeromonas isolation rate and the increase of patient age; the p-value was < 0.001, and the odds ratio was 1.015 (95% confidence interval, 1.013–1.016). Male patients had a significantly higher isolation rate than female patients (0.6% vs. 0.5%); the p-value was < 0.001, and the odds ratio was 1.204 (95% confidence interval, 1.104–1.312).

A) Aeromonas species

![Graph A](image1)

Figure 1. Isolation of Aeromonas species, Campylobacter species and Salmonella species during 2015–2019 from different age groups and gender. The bacterial pathogens were isolated from faecal samples of patients with gastroenteritis. The isolation rates were the positive isolations per 10,000 samples. (A) Aeromonas species, (B) Campylobacter species and (C) Salmonella species. Aeromonas species in human gastroenteritis showed a novel two peaks (20–29 age group and over 80 age group) infection pattern. The isolation of Aeromonas species was positively associated with age (p < 0.001) and significantly higher in male patients than female patients (p < 0.001). Campylobacter species was more isolated in the 10–29 age group; the isolation rate was negatively associated with patient age (p < 0.001) and was significantly higher in male patients than female patients (p < 0.001). Salmonella species was most often isolated in children of 0–4 age group; the isolation rate was negatively associated with patient age (p < 0.001) and was significantly higher in male patients than female patients (p < 0.001).
For *Campylobacters* species isolation, the 10–19- and 20–29-years-old groups had the highest isolate rates, being 472.64 and 458.18, respectively (Figure 1B). There was a significant association between *Campylobacter* species isolation rate and the decrease of patient age; the *p*-value was < 0.001, and the odds ratio was 0.998 (95% confidence interval, 0.998–0.999). Male patients had a significant higher *Campylobacter* isolation rate than female patients (3.97% vs. 2.47%, *p* < 0.001); the odds ratio was 1.617 (95% confidence interval, 1.558–1.679).

For *Salmonella* species isolation, young children (0–4 years old) had the highest isolation rate, being 240.56. The isolation rate gradually decreased as the age increased, with individuals over 80 years old having the lowest isolation rate, being 43.33 (Figure 1C). There was a significant association between *Salmonella* species isolation rate and decreasing age, the *p*-value was < 0.001 and the odds ratio was 0.985 (95% confidence interval, 0.984–0.986). The isolation rates in male and female patients were significantly different (1.59% vs. 1.24%), the *p*-value was < 0.001 and the odds ratio was 1.154 (95% confidence interval, 1.092–1.219).

The positive isolations of *Aeromonas*, *Campylobacter* and *Salmonella* species in individual years, from 2015 to 2019, in different age groups are shown in Table 3.

### Table 3. Isolation of *Aeromonas*, *Campylobacter* and *Salmonella* species in individual years, from 2015 to 2019, in different age groups.

| Age Group (Years) | Aeromonas Positive Case | Campylobacter Positive Case | Salmonella Positive Case | Number of Stool Tested |
|-------------------|-------------------------|----------------------------|--------------------------|------------------------|
|                   | Male | Female | Male | Female | Male | Female | Male | Female |
| 2015              |      |        |      |        |      |        |      |        |
| 0–4               | 8    | 11     | 173  | 109    | 126  | 122    | 6276 | 5075    |
| 5–9               | 11   | 3      | 101  | 56     | 48   | 60     | 2288 | 2285    |
| 10–19             | 26   | 5      | 192  | 87     | 64   | 65     | 2370 | 2463    |
| 20–29             | 15   | 20     | 211  | 210    | 57   | 82     | 2666 | 4681    |
| 30–39             | 18   | 24     | 148  | 179    | 75   | 84     | 3178 | 5316    |
| 40–49             | 23   | 20     | 123  | 120    | 45   | 48     | 2804 | 4675    |
| 50–59             | 14   | 22     | 125  | 136    | 37   | 59     | 2643 | 4488    |
| 60–69             | 15   | 25     | 136  | 140    | 28   | 60     | 2809 | 4861    |
| 70–79             | 15   | 22     | 115  | 103    | 24   | 20     | 2384 | 3751    |
| 80+               | 19   | 41     | 34   | 65     | 18   | 32     | 1812 | 3755    |
| 2016              |      |        |      |        |      |        |      |        |
| 0–4               | 16   | 9      | 131  | 113    | 182  | 165    | 6404 | 5219    |
| 5–9               | 6    | 5      | 103  | 54     | 43   | 48     | 2381 | 2376    |
| 10–19             | 10   | 7      | 168  | 90     | 57   | 59     | 2509 | 2698    |
| 20–29             | 21   | 32     | 147  | 179    | 74   | 105    | 2775 | 5049    |
| 30–39             | 14   | 28     | 130  | 150    | 56   | 84     | 3664 | 5757    |
| 40–49             | 15   | 17     | 129  | 125    | 63   | 62     | 3007 | 5116    |
| 50–59             | 18   | 26     | 116  | 120    | 47   | 61     | 2750 | 4861    |
| 60–69             | 27   | 25     | 121  | 114    | 35   | 44     | 3013 | 5210    |
| 70–79             | 21   | 27     | 101  | 88     | 30   | 52     | 2691 | 4296    |
| 80+               | 24   | 33     | 47   | 52     | 7    | 18     | 2133 | 4379    |
The isolations of *P. shigelloides*, *Shigella* and *Vibrio* species were more common in adults between 20 to 59 years old (Figure 2). The *P. shigelloides* isolation rate was not significantly associated with either gender or age, both had the *p*-values > 0.05. The isolation rate of *Shigella* species was not significantly associated with age, but significantly higher in male than female patients, *p* < 0.001 and odds ratio 2.964 (95% confidence interval, 2.289–3.87). The isolation rate of *Vibrio* species was significantly associated with increasing age, with
The isolations of *P. shigelloides*, *Shigella* species and *Vibrio* species in individual years, from 2015 to 2019, in different age groups and gender. The bacterial pathogens were isolated from faecal samples of patients with gastroenteritis. The isolation rates were the positive isolations per 10,000 samples. (A) *P. shigelloides*, (B) *Shigella* species and (C) *Vibrio* species. The isolations of *P. shigelloides*, *Shigella* and *Vibrio* species were more common during human adult life between 20 to 59 years old. The isolation rate of *Shigella* species was significantly higher in male patients than female patients, *p* < 0.001. The isolation rate of *Vibrio* species was positively associated with age (*p* < 0.001) and significantly higher in male patients than female patients (*p* < 0.001).
Table 4. Isolation of *Plesiomonas shigelloides*, *Shigella* and *Vibrio* species in individual years, from 2015 to 2019, in different age groups.

| Age Group (Years) | Plesiomonas Positive Case | Shigella Positive Case | Vibrio Positive Case | Number of Stool Tested |
|-------------------|---------------------------|------------------------|----------------------|------------------------|
|                   | Male | Female | Male | Female | Male | Female | Male | Female |
| 2015              |      |        |      |        |      |        |      |        |
| 0–4               | 0    | 0      | 1    | 2      | 0    | 0      | 6276 | 5075    |
| 5–9               | 0    | 0      | 1    | 0      | 1    | 0      | 2288 | 2285    |
| 10–19             | 2    | 1      | 0    | 0      | 0    | 0      | 2370 | 2463    |
| 20–29             | 4    | 1      | 2    | 5      | 1    | 0      | 2666 | 4681    |
| 30–39             | 2    | 4      | 3    | 3      | 3    | 2      | 3178 | 5316    |
| 40–49             | 4    | 2      | 8    | 2      | 2    | 1      | 2804 | 4675    |
| 50–59             | 3    | 7      | 6    | 3      | 2    | 1      | 2643 | 4488    |
| 60–69             | 3    | 4      | 2    | 2      | 1    | 0      | 2809 | 4861    |
| 70–79             | 2    | 0      | 0    | 0      | 0    | 1      | 2384 | 3751    |
| 80+               | 1    | 1      | 0    | 1      | 3    | 3      | 1812 | 3755    |
| 2016              |      |        |      |        |      |        |      |        |
| 0–4               | 3    | 0      | 2    | 0      | 0    | 0      | 6404 | 5219    |
| 5–9               | 1    | 2      | 0    | 0      | 0    | 0      | 2381 | 2376    |
| 10–19             | 2    | 1      | 0    | 0      | 3    | 1      | 2509 | 2698    |
| 20–29             | 2    | 8      | 13   | 4      | 1    | 1      | 2775 | 5049    |
| 30–39             | 3    | 7      | 12   | 3      | 3    | 3      | 3664 | 5757    |
| 40–49             | 2    | 5      | 10   | 2      | 2    | 2      | 3007 | 5116    |
| 50–59             | 1    | 2      | 3    | 3      | 3    | 1      | 2750 | 4861    |
| 60–69             | 0    | 3      | 3    | 0      | 3    | 3      | 3013 | 5210    |
| 70–79             | 1    | 0      | 0    | 0      | 1    | 1      | 2691 | 4296    |
| 80+               | 0    | 1      | 0    | 2      | 1    | 1      | 2133 | 4379    |
| 2017              |      |        |      |        |      |        |      |        |
| 0–4               | 0    | 1      | 1    | 1      | 1    | 0      | 5925 | 4990    |
| 5–9               | 0    | 0      | 1    | 1      | 0    | 0      | 2360 | 2344    |
| 10–19             | 1    | 2      | 4    | 0      | 1    | 0      | 2534 | 2622    |
| 20–29             | 2    | 2      | 4    | 1      | 3    | 2      | 2645 | 4862    |
| 30–39             | 1    | 4      | 8    | 3      | 3    | 2      | 3322 | 5488    |
| 40–49             | 7    | 4      | 5    | 4      | 2    | 2      | 2981 | 4996    |
| 50–59             | 6    | 5      | 4    | 2      | 0    | 2      | 2780 | 4720    |
| 60–69             | 2    | 3      | 2    | 1      | 2    | 0      | 2934 | 5073    |
| 70–79             | 4    | 2      | 0    | 1      | 0    | 1      | 2778 | 4715    |
| 80+               | 0    | 2      | 0    | 0      | 1    | 1      | 2409 | 4720    |
Table 4. Cont.

| Age Group (Years) |  |  |  |  |  |  |  |  |  |  |
|------------------|--|--|--|--|--|--|--|--|--|--|
|                  | Plesiomonas Positive Case | Shigella Positive Case | Vibrio Positive Case | Number of Stool Tested |
|                  | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| 2018             |  |  |  |  |  |  |  |  |  |  |
| 0–4              | 0   | 0   | 2   | 0   | 0   | 0   | 5619 | 4580 |  |  |
| 5–9              | 0   | 2   | 1   | 0   | 1   | 0   | 2224 | 2312 |  |  |
| 10–19            | 0   | 1   | 0   | 0   | 1   | 0   | 2770 | 2856 |  |  |
| 20–29            | 2   | 3   | 4   | 2   | 1   | 1   | 3014 | 5072 |  |  |
| 30–39            | 7   | 1   | 12  | 5   | 0   | 2   | 3533 | 5639 |  |  |
| 40–49            | 2   | 2   | 0   | 3   | 1   | 1   | 2994 | 5032 |  |  |
| 50–59            | 2   | 9   | 4   | 4   | 1   | 1   | 2775 | 4734 |  |  |
| 60–69            | 2   | 6   | 4   | 4   | 0   | 1   | 2862 | 5177 |  |  |
| 70–79            | 3   | 2   | 2   | 1   | 1   | 1   | 2883 | 4687 |  |  |
| 80+              | 0   | 1   | 1   | 0   | 2   | 0   | 2217 | 4234 |  |  |
| 2019             |  |  |  |  |  |  |  |  |  |  |
| 0–4              | 0   | 2   | 0   | 0   | 0   | 0   | 5949 | 4960 |  |  |
| 5–9              | 0   | 1   | 0   | 0   | 0   | 0   | 2293 | 2318 |  |  |
| 10–19            | 2   | 0   | 3   | 2   | 0   | 0   | 2838 | 3020 |  |  |
| 20–29            | 5   | 8   | 10  | 7   | 2   | 2   | 2950 | 5048 |  |  |
| 30–39            | 3   | 6   | 19  | 3   | 1   | 1   | 3624 | 5942 |  |  |
| 40–49            | 2   | 4   | 7   | 2   | 3   | 1   | 3254 | 5134 |  |  |
| 50–59            | 3   | 4   | 6   | 1   | 2   | 3   | 2862 | 4755 |  |  |
| 60–69            | 4   | 3   | 1   | 2   | 2   | 0   | 3096 | 5310 |  |  |
| 70–79            | 1   | 1   | 1   | 1   | 2   | 2   | 2982 | 5059 |  |  |
| 80+              | 1   | 1   | 0   | 0   | 0   | 0   | 2326 | 4555 |  |  |

3.5. Isolations of Aeromonas, Salmonella and Vibrio Species Were Positively Associated with Increase of Temperature

There was a strong positive correlation between *Aeromonas* species isolation rate and the local monthly mean maximum temperatures (\( p < 0.001 \), the Spearman’s rank correlation coefficient = 0.807). *Aeromonas* species were more often isolated in January, February, March and April each year. The isolation rates started to decrease in May and maintained at low isolation rates from June to November. In December, *Aeromonas* isolation started to increase again, corresponding to the onset of the Australian summer (Figure 3A).
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The isolation rate of Campylobacter species isolation was not significantly associated with the change of temperature, $p = 0.835$ and the Spearman's rank correlation coefficient was $-0.027$ (Figure 3B). The isolation of Salmonella species was also positively associated with temperature, with $p < 0.001$ and the Spearman’s rank correlation coefficient 0.539 (Figure 3C).

**Figure 3.** Isolation of Aeromonas species, Campylobacter species and Salmonella species in individual month during 2015–2019. The bacterial pathogens were isolated from faecal samples of patients with gastroenteritis. The isolation rates were the positive isolations per 10,000 samples. The mean maximum temperature was from Observatory Hill, Sydney. (A) Aeromonas species, (B) Campylobacter species and (C) Salmonella species. There was a strong positive correlation between Aeromonas species isolation rate and the local monthly mean maximum temperatures ($p < 0.001$, the Spearman’s rank correlation coefficient = 0.807). Aeromonas species were more often isolated in January, February, March and April each year. The isolation rates started to decrease in May and maintained at low isolation rates from June to November. In December, Aeromonas isolation started to increase again (Figure 3A). The isolation rate of Campylobacter species isolation was not significantly associated with the change of temperature, $p = 0.835$ and the Spearman’s rank correlation coefficient was $-0.027$ (Figure 3B). The isolation of Salmonella species was also positively associated with temperature, with $p < 0.001$ and the Spearman’s rank correlation coefficient 0.539 (Figure 3C).
The isolation rate of *Campylobacter* species was not significantly associated with the change of temperature, $p = 0.835$ and the Spearman’s rank correlation coefficient was $-0.027$ (Figure 3B). In contrast, the isolation of *Salmonella* species was also positively associated with temperature, with $p < 0.001$ and the Spearman’s rank correlation coefficient 0.539 (Figure 3C). There were no significant associations between the isolations of *P. shigelloides* and *Shigella* species with temperature. The isolation of *Vibrio* species showed a moderate positive correlation with temperature, $p = 0.013$ and the Spearman’s rank correlation coefficient 0.319 (Figure 4).

**Figure 4.** Isolation of *Plesiomonas shigelloides*, *Shigella* species and *Vibrio* species in individual month during 2015–2019. The bacterial pathogens were isolated from faecal samples of patients with gastroenteritis. The isolation rates were the positive isolations per 10,000 samples. The mean maximum temperature was from Observatory Hill, Sydney. **(A)** *P. shigelloides*, **(B)** *Shigella* species and **(C)** *Vibrio* species. There were no significant associations between the isolations of *P. shigelloides* and *Shigella* species with temperature. The isolation of *Vibrio* species was positively associated with temperature ($p = 0.013$).
3.6. The Identities of Isolated Aeromonas Species

A subset (48 isolates) of isolated Aeromonas species was identified to the species level, using MLPA. Five Aeromonas species were identified, of which 25 isolates were Aeromonas veronii (25%), 13 isolates were Aeromonas caviae (27%), six isolates were Aeromonas hydrophila (12.5%), two isolates were Aeromonas dhakensis (4.2%) and two isolates were Aeromonas rivipollensis (4.2%) (Figure 5).

Figure 5. Identification of Aeromonas species through use of multilocus phylogenetic analysis. Aeromonas isolates were subjected to species identification based on the sequences of seven housekeeping genes, namely gyrB, rpoD, gyrA, recA, dnaJ, dnaX and atpD. Bootstrap values were generated from 1000 replicates. Bootstrap values between 0.7 and 1 are shown. Of the 48 Aeromonas isolates (blue) subjected to analysis, 25 isolates were Aeromonas veronii, 13 isolates were Aeromonas caviae, six isolates were Aeromonas hydrophila, two isolates were Aeromonas dhakensis and two isolates were Aeromonas rivipollensis.
4. Discussion

In this study, we examined the infection of *Aeromonas* species and an additional five enteric bacterial pathogens in human gastroenteritis by analysis of bacterial pathogen cultivation data from 375,842 stool samples processed in a large Australian diagnostic laboratory in five consecutive years, from 2015 to 2019. We also identified a subset of *Aeromonas* isolates to species level.

We found that *Aeromonas* species were the third most common bacterial pathogens in human gastroenteritis (Table 1). *Aeromonas* species were the only isolated bacterial pathogens in most patients with positive isolation, supporting their role in causing human gastroenteritis (Table 2). *Campylobacter* and *Salmonella* species were the top two most common bacterial pathogens causing human gastroenteritis, and most *Salmonella* species were non-typhoidal species (Table 1). Other enteric bacterial pathogens, such as *P. shigelloides*, *Shigella* and *Vibrio* species, were also isolated; the infections of these bacterial species in human gastroenteritis were much less common in comparison to *Campylobacter*, *Salmonella* and *Aeromonas* species (Table 1).

*Aeromonas* isolation rate was significantly correlated with increasing age. *Aeromonas* species’ isolation rate was low in young children, and it gradually increased as children got older. It reached the first small peak in the young-adult group of 20–29 years old and maintained a steady isolation rate in human adult life. Interestingly, the *Aeromonas* species isolation rate started to increase again in individuals over 50 years old, including the second peak in the oldest age group (more than 80 years old group) (Figure 1A and Table 3). This interesting isolation pattern was not seen in the other five bacterial pathogen species analysed in this study. *Campylobacter* and *Salmonella* species infections in human gastroenteritis were also age related, but their most commonly affected age groups were different from *Aeromonas* species infection. *Campylobacter* species more often affected individuals between 10 to 29 years old (Figure 1B), and *Salmonella* species most commonly affected young children (Figure 1C). The other three enteric pathogens, *P. shigelloides*, *Shigella* and *Vibrio* species, were mostly isolated from individuals of 20 to 59 years old (Figure 2 and Table 4). The novel infection pattern of *Aeromonas* in human gastroenteritis suggests that the two peaks are likely due to different reasons. The first peak most likely was due to increased exposure to the sources of infection. The continuous increase of *Aeromonas* infection in individuals over 50 years old, including the second peak, most likely was due to decreased host defence associated with human aging.

*Aeromonas* species infection detected in this study was predominantly locally acquired, with less than 5% of patients having a documented travel history. Previous studies have reported isolation of *Aeromonas* species from various sources, such as drinking water, fish, vegetables and freshwater sources, such as lakes and rivers [28]. The sources of *Aeromonas* species infection in Australian patients with gastroenteritis remain to be investigated. *Aeromonas* species associated gastroenteritis occurred more often in warm seasons, showing that the main infectious sources for human infections, once identified, should be particularly monitored for *Aeromonas* species load during warm seasons (Figure 3). The isolations of *Aeromonas*, *Campylobacter*, *Salmonella*, *Shigella* and *Vibrio* species were significantly higher from male patients than female patients, reflecting potentially a gender-based difference in lifestyle.

Five *Aeromonas* species were identified in our study for 48 *Aeromonas* isolates, and *A. veronii* was the most common species (52%), followed by *A. caviae* (27%) and *A. hydrophila* (12.5%) (Figure 5). In a recent study examining antibiotic resistance of *Aeromonas* species isolated from different infection sites, Sinclair et al. included 18 *Aeromonas* species isolated from stool samples in Queensland, Australia, of which 12 isolates (66.7%) were *A. veronii* and four isolates (22.2%) were *A. caviae* [29]. Our data and the study by Sinclair et al. consistently showed that *A. veronii* was the most common *Aeromonas* species isolated from stool samples of patients with gastroenteritis in the Australian population. In a review paper, Fernandez-Bravo et al. combined *Aeromonas* species isolated from faecal samples in different studies, showing that *A. caviae* had the highest isolation (49%), followed by
A. veronii (25%), suggesting that the most common Aeromonas species isolated from stool samples in different regions vary [1]. Our study is also the first reporting Aeromonas rivipollensis isolation from human faecal samples. A. rivipollensis was previously isolated from river sediments [30].

In summary, this study found that Aeromonas species were the third most common enteric bacterial pathogen in Australian patients with gastroenteritis, following Campylobacter and Salmonella species. Aeromonas infection in patients with gastroenteritis was significantly correlated with increasing age, more often occurred in warm seasons and in male than female patients. A. veronii was the most common Aeromonas species isolated from patients with gastroenteritis in the Australian population. Aeromonas species infection detected in this study was predominantly locally acquired. In conclusion, the findings from this study support the importance of Aeromonas species in human gastroenteritis and suggest that the sources of Aeromonas infection in Australian patients should be further investigated.

**Author Contributions:** C.Y. analysed the data. C.Y. and L.Z. played a major role in writing the manuscript. M.C.W. obtained the original diagnostic data and provided critical feedback to the manuscript. F.L. identified Aeromonas species, using MLPA. S.M.R. conceived Aeromonas species identification, using MLPA, with L.Z. and provided critical feedback to the manuscript. L.Z. and M.C.W. conceived the project. All authors have read and agreed to the published version of the manuscript.

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