

1 *For publication in: Journal of Clinical Microbiology (Full-Length Paper)*

2

3 Proficiency of WHO Global Foodborne Infections Network External Quality Assurance System
4 participants in the identification and susceptibility testing of thermo-tolerant *Campylobacter* spp.
5 from 2003-2012.

6

7 Susanne Karlsmose Pedersen^{1*}, Jaap A. Wagenaar^{2,3,4}, Håkan Vigre¹, Louise Roer¹, Matthew
8 Mikoleit^{5#a}, Awa Aidara-Kane⁶, Amy L. Cawthorne⁶, Frank M. Aarestrup¹, and Rene S.
9 Hendriksen¹

10

11 ¹WHO Collaborating Centre for Antimicrobial Resistance in Food borne Pathogens and European
12 Union Reference Laboratory for Antimicrobial Resistance, National Food Institute, Technical
13 University of Denmark, Kgs. Lyngby, Denmark

14

15 ²Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht
16 University, Yalelaan 1, 3584 CL Utrecht, The Netherlands

17

18 ³WHO Collaborating Center for *Campylobacter*/OIE Reference Laboratory for Campylobacteriosis,
19 Utrecht/Lelystad, The Netherlands

20

21 ⁴Wageningen Bioveterinary Research, Lelystad, the Netherlands

22

23 ⁵WHO Collaborating Centre for Surveillance, Epidemiology and Control of *Salmonella* and other
24 Foodborne Diseases; Division of Foodborne, Waterborne, and Environmental Diseases; National

25 Center for Emerging and Zoonotic Infectious Diseases; Centers for Disease Control and Prevention,
26 Atlanta, Georgia, USA

27

28 ^{#a} Current Address: Division of Global Health Protection, Center for Global Health, Centers for
29 Disease Control and Prevention, Atlanta, Georgia, USA

30

31 ⁶World Health Organization, Department of Food Safety, Zoonoses and Foodborne Diseases,
32 Geneva, Switzerland

33

34

35 *: Corresponding author: Susanne Karlsrose Pedersen

36 National Food Institute, Technical University of Denmark, Kemitorvet, Building 204, DK-2800

37 Kgs. Lyngby, Denmark

38 Phone: +45 35 88 60 00

39 E-mail: suska@food.dtu.dk

40

41 Running title: Proficiency test of *Campylobacter*

42

43 Key words: World Health Organization, Global Foodborne Infections Network, proficiency test,
44 quality assurance, *Campylobacter jejuni*, *Campylobacter coli*, identification, antimicrobial
45 susceptibility testing,

46

47

48

49 **Abstract**

50 *Campylobacter* spp. are food- and water borne pathogens. While rather accurate estimates for these
51 pathogens are available in industrialized countries, a lack of diagnostic capacity in developing
52 countries limits accurate assessments of prevalence in many regions. Proficiency in the
53 identification and susceptibility testing of these organisms is critical for surveillance and control
54 efforts. The aim of the study was to assess performance for identification and susceptibility testing
55 of thermo-tolerant *Campylobacter* among laboratories participating in the World Health
56 Organization (WHO) Global Foodborne Infections Network (GFN) External Quality Assurance
57 System (EQAS) over a nine year period.
58 Participants (primarily national level laboratories) were encouraged to self-evaluate performance as
59 part of continuous quality improvement.
60 The ability to correctly identify *Campylobacter* spp. varied by year and ranged from 61.9 % (2008)
61 to 90.7 % (2012), and the ability to correctly perform antimicrobial susceptibility testing (AST) for
62 *Campylobacter* spp. appeared to steadily increase from 91.4 % to 93.6 % in the test period (2009-
63 2012).
64 Poorest performance (60.0 % correct identification and 86.8 % correct AST results) was observed in
65 African laboratories.
66 Overall, approximately 10 % of laboratories reported either an incorrect identification or
67 antibiogramme. As most participants were (supra)-national reference laboratories, these data raise
68 significant concerns regarding capacity and proficiency at the local, clinical level. Addressing these
69 diagnostic challenges is critical for both patient level management and broader surveillance and
70 control efforts.

71

72

73 **Introduction**

74 Campylobacteriosis in humans is typically presents as acute diarrhea with fever. However, more
75 significant sequelae such as Guillain-Barré syndrome (GBS), reactive arthritis (ReA) and irritable
76 bowel syndrome (IBS) have been reported following *Campylobacter* gastroenteritis (4).

77 Most human cases are caused by thermo-tolerant *Campylobacter* spp. which are zoonotic bacteria
78 found in animals such as poultry, cattle and pigs as well as contaminants of various foodstuffs and
79 water (1, 2).

80

81 *Campylobacter jejuni* or *Campylobacter coli* are the most commonly implicated species and
82 campylobacteriosis is the most frequently reported bacterial foodborne illness in most developed
83 countries. However, data from developing countries data is often limited by a lack of diagnostic
84 capacity (1, 3, 4).

85 Antimicrobials are typically not indicated for mild/moderate enteritis in otherwise healthy
86 individuals. However, antimicrobial therapy may be warranted for severe or bloody diarrhea.
87 Antimicrobials are also used in the management of extra-intestinal (invasive) infections. The
88 macrolides (e.g. erythromycin or azithromycin) are commonly used for empiric treatment of
89 campylobacteriosis and fluoroquinolones (e.g. ciprofloxacin) may be a second line therapy for
90 adults. Accurate antimicrobial susceptibility testing is critical for developing empiric therapy
91 guidelines and monitoring emerging resistance. Increasing antimicrobial resistance (AMR),
92 especially multidrug resistance to fluoroquinolones and azithromycin significantly limits treatment
93 options for severe/invasive disease. Access to last line drugs such as carbapenems is often beyond
94 the reach of many in the developing world (5).

95

96 Since 2000, the World Health Organization (WHO) Global Foodborne Infections Network (GFN)
97 (formerly WHO Global Salm-Surv (WHO GSS)) has functioned as an international platform to
98 enhance the capacity of countries to detect, control and prevent foodborne and other enteric
99 infections. Part of this capacity building work has focused on identification and susceptibility
100 testing of *Campylobacter*. Since 2000, WHO GFN has offered members the opportunity to
101 participate at no cost in an annual External Quality Assurance System (EQAS). Although the
102 primary focus of the EQAS is serotyping and antimicrobial susceptibility testing (AST) of
103 *Salmonella*; identification and AST of *Campylobacter* is included as a separate module.
104 Laboratories may choose to participate in all or some components. Approximately 200 laboratories
105 participate in one or more components of the EQAS. Of these, approximately 25 % will participate
106 in the *Campylobacter* module. The WHO GFN program focuses activities mainly at reference level
107 facilities (supranational, national, or subnational). While some of these facilities may perform
108 clinical testing; clinical diagnostic laboratories typically do not participate in EQAS (6,7).
109 Participants report results electronically and receive their results immediately. Participants are
110 encouraged to utilize their results as part of continuous quality improvement.

111

112 The aim of this paper is to summarise and describe temporal and geographic trends in the
113 performance of the *Campylobacter* component of the EQAS (identification and AST) observed
114 between 2003-2012.

115

116 **Materials and Methods**

117 The *Campylobacter* identification component has included *C. jejuni* and *C. coli* since 2003 and
118 *Campylobacter lari* (from 2003-2008). AST of *C. jejuni* and *C. coli* have been included since 2009.
119 Due to the limited availability of epidemiological cut off values (ECOFFs) for other *Campylobacter*

120 spp., the AST component, only includes *C. jejuni* and *C. coli*. Since 2003, the Technical University
121 of Denmark, National Food Institute (DTU Food) in collaboration with members of the WHO GFN
122 steering committee have organized this proficiency test annually (except 2005). DTU Food
123 coordinated the selection of test strains and verified the identification and AST of test strains.
124 Results obtained by DTU-Food were reconfirmed in a blinded manner by a referee laboratory
125 (United States' Centers for Disease Control and Prevention (CDC)). Further details on the
126 preparatory work and the EQAS-setup are described in the annual EQAS reports available on the
127 Internet (<http://www.who.int/gfn/activities/eqas/en/>).

128
129 While the target audience for the EQAS is national public health, food and veterinary reference
130 laboratories, in special instances (particularly in countries without a designated referral laboratory)
131 the organizers occasionally permit a select number of clinical and/or research laboratories to
132 participate in the EQAS. EQAS participants have the option to participate in all or some of the
133 components. Participants in the WHO GFN proficiency test for identification and/or AST of
134 *Campylobacter* receive two vials each containing a lyophilized *Campylobacter* isolate (challenge
135 strains). In addition, all laboratories participating in the *Campylobacter* AST component were
136 provided an isolate of *C. jejuni* ATCC 33560 upon request. Protocols available on the Internet
137 (<http://www.antimicrobialresistance.dk/233-169-215-eqas.htm>) described how to revive and test the
138 isolates and referred to a manual on sub-culturing and maintenance of quality control (QC) strains.

139
140 For the identification component, the protocol specified that the laboratory's routine methods
141 should be applied. Laboratories were free to utilize conventional phenotypic identification,
142 molecular identification, or a combination of methods for identification. Laboratories who

143 participated only in the AST component (did not perform identification), were provided upon
144 request the identification of the coded *Campylobacter* strains.

145 While multiple methods were used for identification; during this period, time validated methods for
146 susceptibility testing of *Campylobacter* by disk diffusion or E-test were not internationally
147 available. As such only broth or agar dilution methods (MIC) were accepted for the AST
148 component. Epidemiological cut-off values (ECOFFs) for the interpretation of disk diffusion zones
149 for ciprofloxacin, erythromycin and tetracycline against *C. coli* as well as ciprofloxacin,
150 erythromycin, tetracycline, and gentamycin for *C. jejuni* are now incorporated into EUCAST
151 guidelines Participants could test and submit results for chloramphenicol (CHL), ciprofloxacin
152 (CIP), erythromycin (ERY), gentamicin (GEN), nalidixic acid (NAL), streptomycin (STR), and
153 tetracycline (TET). The protocol listed the interpretative criteria applied for this EQAS, i.e.
154 ECOFFs according to EUCAST (<http://www.eucast.org>) which allowed two categories of
155 characterization (resistant [non-wildtype], R or susceptible [wildtype], S) for the *C. jejuni* and *C.*
156 *coli* test strains.

157
158 The WHO GFN EQAS was set-up as a self-evaluating system in which participants directly upon
159 submission of results received a report comparing their obtained results to quality assured and
160 verified expected results and itemizing the laboratory's eventual deviations. Deviations for the
161 identification component were reported as incorrect results and no attempt was made to quantify
162 their severity. For the AST component, the acceptance limit was set at 5 % deviations, i.e. one
163 deviation would categorize the laboratory's results as unacceptable. The analysis was based on
164 assigning all results the same level of influence, i.e. disregarding the impact of the variation in the
165 selection of test strains from year to year (the susceptibility testing of some strains could be more

166 difficult than others) and the varying participation levels (some years, more laboratories participated
167 compared to other years).

168

169 For each of the world regions, the annual proportion of correctly identified species were analyzed
170 for i) significant variation between the years 2003 to 2012 using the function `Fisher.test` in R and ii)
171 a time-trend from 2003 to 2012 by performing a chi-square test for trend in proportions using the
172 function `prop.trend.test` from the R-package `stats`. Next, for each region, the data was aggregated to
173 the overall proportion of correctly identified *Campylobacter* species over the whole period from
174 2003 to 2012. These data were used to test if the proportion of correctly identified species was
175 different between the regions using the `prop.test` function in the R-package `stats`.

176

177 To assess potential differences between regions in the AST performance, the proportion of correct
178 AST result for each antibiotic (CHL, CIP, ERY, GEN, NAL, STR and TET) was analyzed for
179 significant differences between regions using the `prop.test` function in the `stat` R-package `stats`.

180

181 All presented 95 % confidence intervals for the proportions were obtained using the function
182 `binconf` in the R-package `Hmisc`.

183

184 **Results**

185 In total, laboratories from 96 countries (Figure 1) participated in the *Campylobacter* identification
186 and/or AST component of the EQAS in one or more iterations from 2003 to 2012 and included
187 national and other reference laboratories from the veterinary, food and public health sector.

188

189 For the identification component of the EQAS, the number of countries participating each year
190 were: 53 (2003), 62 (2004), 59 (2006), 59 (2007), 63 (2008), 54 (2009), 62 (2010), 57 (2011) and
191 69 (2012). In some cases multiple laboratories from a country participated in the EQAS (e.g. MoH
192 and MoA laboratories). The cumulative number of laboratories participating in the *Campylobacter*
193 module was: 97, 111, 100, 104, 112, 92, 100, 82 and 112 participants each of the years,
194 respectively.

195

196 For the AST of *Campylobacter* in the years 2009, 2010, 2011 and 2012, the numbers of
197 participating countries from each region were: Africa (2, 2, 7, 4); Asia & Middle East (1, 1, 1, 3);
198 Caribbean (0, 0, 0, 1); Europe (7, 9, 7, 11); Latin America (4, 7, 6, 6); North America (1, 2, 2, 2);
199 Oceania (0, 0, 1, 0); Russian region (0, 1, 1, 0); Southeast Asia (4, 5, 4, 6). The cumulative number
200 of laboratories participating from each country was: 25, 37, 38 and 47 participants in total each of
201 the years, respectively. In all, 18 laboratories participated twice, 14 participated three times and 11
202 participants took part in all four *Campylobacter* AST iterations from 2009 to 2012.

203

204 The overall ability to correctly identify *Campylobacter* spp. fluctuated over the years 2003 to 2012
205 (Figure 2), and when focusing at *C. coli* and *C. jejuni* between the regions, a significant difference
206 could be identified in Europe and Latin America in the proportion of correctly identified
207 *Campylobacter* species between years (data not shown). No time-related trend was identified in
208 Europe nor Latin America, and other factors (such as new laboratories joining or previous
209 participants leaving the program) likely contributed to this variation. Significant variation between
210 the years was not found in any other regions.

211

212 There was a strong significant difference between the regions as to the proportion of correctly
213 identified *Campylobacter* species, with Oceania and North America exhibiting the highest, and
214 Africa and the Caribbean the lowest proportions of correctly identified *Campylobacter* species
215 (Figure 3). It is important to consider that in some regions (e.g. Oceania), participation was limited
216 (n=1) and may fail to truly reflect regional capacity.

217
218 In the iterations from 2003 to 2012, identifications were correctly performed between 59.3 % (2011;
219 *C. coli*; N = 81) and 96.4 % (2012; *C. jejuni*; N = 112) with an average over the years of 79.3 %
220 (based on the result of two isolates per year, i.e. 18 isolates in total, of *C. jejuni*, *C. coli* and *C. lari*;
221 total number of observations, N = 1,736). Comparing between the species, it appears that
222 participants are able to correctly identify *C. jejuni*, (90.4 % correct) than *C. coli* and *C. lari* (74.1 %
223 and 68.8 % correct identifications, respectively). This result is not unexpected as typical *C. jejuni* is
224 readily identified by hippurate hydrolysis whereas other species require additional, more complex
225 tests.

226
227 Subsequent to the validation of the submitted data, 1,565 AST results could be included for analysis
228 for the eight *Campylobacter* isolates included in the four iterations from 2009 to 2012. Of these, 7.3
229 % deviated from the expected. For all antimicrobials included in the AST performance test, there
230 was a significant difference between the performance of the regions, where Europe exhibited the
231 highest correspondence with the expected results and Southeast Asia the lowest (Figure 4).

232
233 Deviations appeared to be in particular caused by streptomycin with a deviation level at 11.5 %, but
234 also for erythromycin, tetracycline and ciprofloxacin, fairly high deviation levels were seen (8.5 %,
235 8.1 %, and 6.4 % respectively). In particular, one bacterial strain caused a high level of deviations,

236 i.e. WHO 2009 C-9.2 (resistant to CIP, NAL, and TET) for which 11.3 % of the submitted results
237 deviated from the expected. A low deviation level (4.2 %) was obtained for tests towards
238 chloramphenicol, towards which all the test strains were susceptible. A comparison of the obtained
239 results from all laboratories which participated in the AST component in one or more of the four
240 iterations, indicated a slight increase in performance (Figure 5). Disregarding results from the
241 Caribbean and Oceania due to the limited number of submitted results, the summary of the four
242 years' results per region provided an indication of a generally low performance of the participants
243 in the Southeast Asian region and the African region, with levels at 17.0 % and 13.2 % deviations,
244 respectively. In 2012, however, all regions except Southeast Asia (deviation level at 14.2 %)
245 exhibited deviation levels lower than 10 % (the Russian region did not participate in the 2012
246 iteration).

247
248 In the four iterations, 51 (75 %) laboratories uploaded one or more values for the QC reference
249 strain, *C. jejuni* ATCC 33560 suggesting that 25 % of the labs did not test QC strain for reference.
250 Of the submitted values for the QC reference strain, an average of 17.2 % were out of the QC range
251 when evaluated towards one of the validated methods described by CLSI (e.g. VET01-A4) (8).
252 Analysis of regional differences in this context reveals Africa as the region with the highest level of
253 laboratories submitting AST results for the *Campylobacter* test strains without submitting results
254 for the *C. jejuni* ATCC 33560 reference strain.

255

256 **Discussion**

257 The proficiency test results are intended to be utilized for continuous quality improvement.
258 However, some participating laboratories did not demonstrate improvement in identification or AST
259 over time. Information about corrective actions implemented in the individual laboratories based on

260 the deviations in their evaluation report could have added to the analysis but was unfortunately not
261 available. This proficiency test shows a worrisome tendency where even national reference
262 laboratories in regions, normally anticipated to perform flawless, have approximately 10 %
263 incorrect results in both tests. Given the complex microbiology of *Campylobacteriaceae* and the
264 fastidious nature of these organisms, when these results are extrapolated to lower level facilities
265 such as clinical laboratories, the ability to correctly identify and antimicrobial susceptibility test
266 *Campylobacter* is likely significantly higher than the 10 % error observed among participating
267 laboratories. In addition, *Campylobacter* results were only received from ~25 % of the total number
268 of participating laboratories in the WHO GFN EQAS. This suggests that nearly 75 % of participants
269 lack basic capacity for testing *Campylobacter*. These findings are worrying in light of the
270 importance of *Campylobacter* as a foodborne pathogen. Thus, actions are still required to improve
271 the performance of laboratories and report correct data on *Campylobacter* worldwide.

272
273 Performance (pass/fail) criteria were not specified for the *Campylobacter* identification module,
274 though the average of 79.3 % correctly identified strains indicates that some laboratories would
275 need to assess their routine to improve their performance. Assessing the general performance of the
276 AST component of all participating laboratories over the four years, the average deviation level at
277 7.3 % exceeds the defined acceptance limit of 5 %. The development in the annual deviation levels;
278 however, could not indicate a trend with statistical significance. For the AST, the high level of
279 incorrectly reported results is critical, especially for macrolides (8.5 %) and fluoroquinolones (6.4
280 %), since these two antimicrobials classes are the preferred choice for treatment.

281
282 The frequent incidence of *Campylobacter* diarrhea, increasing drug resistance, and the potential for
283 long term sequelae, highlight the importance of accurately understanding the socio-economic

284 burden of campylobacteriosis (4). Increased competence of reference laboratories for identification
285 and AST *C. jejuni* and *C. coli* therefore support disease surveillance and control programs.
286 However, the ability of a referral center to impact surveillance is contingent upon an
287 isolate/specimen flow from lower level laboratories. On average, only 25 % of EQAS participants
288 reported results for *Campylobacter*, suggesting widespread gaps in capacity. The advancement in
289 whole genome sequencing and *in silico* bioinformatics tools combined with lower prices, is a
290 promising development in enhancing the ability of national reference laboratories to correctly
291 identify and susceptibility testing *Campylobacter* in the future. Similarly, molecular assays and
292 other culture independent tests may increase surveillance capacity at the clinical level. While these
293 technologies currently are not a substitute for culture, they may provide estimates of burden and
294 help determine which specimens should be subjected to conventional culture.

295
296 The self-evaluation design of this proficiency test was intended to challenge the participating
297 laboratories to assess their current identification and AST methods for *Campylobacter* also allowing
298 them to include the proficiency test outcome as an external quality assessment of the relevant
299 methods. Self-evaluation is a concept well-known to laboratories following a quality assurance
300 standard requiring quality control procedures e.g. ISO/IEC 17025 (9) and might include monitoring
301 the validity of test results by regular use of internal quality control using reference materials or
302 participation in proficiency-testing programmes. Apart from the obvious connection to the WHO
303 GFN EQAS as a proficiency test, laboratories participating in the programme are offered material
304 for internal control in the form of both the certified ATCC reference strain, *C. jejuni* ATCC 33560,
305 and the test strains which can be regarded as internal control strains and consequently should be
306 stored and maintained.

307

308 In addition to self-evaluation, the possibility of introducing approaches like mentoring of
309 participants, training courses, and E-learning could be explored and suggested to the participants.
310 The question of resources must, however, be considered, for example, mentoring of participants
311 appears to be a rewarding but also is a resource demanding approach of capacity building Regional
312 follow-up is likely to be a rewarding approach and should be based on evaluation of regional needs
313 and challenges. Especially for the African region and Southeast Asia, it appears that specific follow-
314 up is required. For example, the submitted results for the AST component indicate that many
315 laboratories did not perform adequate internal quality control (17.2 % of submitted results for the
316 ATCC reference strain were out of range), which is why WHO GFN capacity building efforts focus
317 at encouraging the maintenance of relevant quality assurance as part of the laboratory routines.
318 Internal laboratory QA ensures minimization of variable factors influencing the obtained result for a
319 test strain. These factors include the media content, the activity of the antimicrobial, and the testing
320 of a QC reference strain according to an internationally recognized standard (e.g. CLSI).
321 Laboratories that have introduced relevant quality assurance of the variable factors facilitate a good
322 performance. For all laboratories performing AST of *Campylobacter* species, testing of the *C. jejuni*
323 reference strain (ATCC33560) should be a routine QA measure providing quality control for both
324 the method and the reagents. Moreover, results outside the quality control ranges should always
325 induce appropriate follow-up.

326

327 In conclusion, this annually provided proficiency test supports the identification and AST of
328 *Campylobacter* and allows national reference laboratories free of charge to evaluate their obtained
329 results by comparison to quality assured and verified expected results. Overall, we found that global
330 ability to correctly identify *Campylobacter* spp. fluctuated over the years up to 90.7 % and the
331 ability to correctly perform AST appeared to steadily increase to 93.6 %. African laboratories

332 followed by Southeast Asian had the lowest performance in both identifying the *Campylobacter*
333 spp. conducting AST. Our results reveal a worrisome tendency where approximately 10 % of
334 laboratories report either an incorrect diagnosis or antimicrobial susceptibility profile for treatment.
335 This will compromise the ability to correctly diagnose illness, effectively treat patients and will
336 provide unreliable data for pathogen and AST surveillance systems if not attended.

337

338 **Acknowledgement**

339 This work was funded by the World Health Organization Global Foodborne Infections Network
340 (<http://www.who.int/gfn>) and by COMPARE, which has received funding from the European
341 Union's Horizon 2020 research and innovation programme under grant agreement No 643476.
342 Thanks to Arne B. Jensen for setting up the submission database for capturing and evaluating the
343 results of the EQAS participants.

344

345 **Reference List**

- 346 1. EFSA and ECDC [European Food Safety Authority, European Centre for Disease
347 Prevention and Control]; The European Union Summary Report on Trends and Sources of
348 Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010; EFSA Journal 2012;
349 10(3):2597. [442pp.] doi:10.2903/j.efsa.2012.2597. Available online:
350 <http://www.efsa.europa.eu/efsajournal>
- 351 2. Jacobs-Reitsma, W., U. Lyhs, J. Wagenaar. 2008. *Campylobacter* in the Food Supply, In:
352 *Campylobacter* 3rd edition, (Eds.) Nachamkin, I., Szymanski C.M., and Blaser, M.J., ASM
353 Press, Washington, D.C. pp. 427-444.

- 354 3. FAO/WHO [Food and Agriculture Organization of the United Nations/World Health
355 Organization]. 2009. Risk assessment of *Campylobacter* spp. in broiler chickens:
356 Interpretative Summary. Microbiological Risk Assessment Series No 11. Geneva. 35pp.
- 357 4. The global view of campylobacteriosis: report of an expert consultation, Utrecht,
358 Netherlands, 9-11 July 2012. ISBN 978 92 4 156460 1 (accessed via www.who.int on March
359 22nd 2017)
- 360 5. Engberg J, Aarestrup FM, Taylor DE, Gerner-Smidt P, Nachamkin I. 2001. Quinolone and
361 Macrolide Resistance in *Campylobacter jejuni* and *C. coli*: Resistance Mechanisms and
362 Trends in Human Isolates. EID Vol. 7, 24-34.
- 363 6. Hendriksen, R.S., M. Mikoleit, V.P. Carlson, S. Karlslose, A.R. Vieira, A.B. Jen sen, A.M.
364 Seyfarth, S.M. DeLong, F.-X. Weill, D.M.A. Lo Fo Wong, F.J. Angulo, H.C. Wegener and
365 F.M. Aarestrup. 2009a. WHO Global Salm-Surv External Quality Assurance System for
366 Serotyping of *Salmonella* Isolates from 2000 to 2007. J Clin Microbiol 47, 2729–2736.
367 doi:10.1128/JCM.02437-08
- 368 7. Hendriksen, R.S., A.M. Seyfarth, A.B. Jensen, J Whichard, S. Karlslose, K. Joyce, M.
369 Mikoleit, S.M. Delong, F.-X. Weill, A. Aidara-Kane, D.M.A. Lo Fo Wong, F.J. Angulo,
370 H.C. Wegener, F.M. Aarestrup. 2009b. Results of Use of WHO Global Salm-Surv External
371 Quality Assurance System for Antimicrobial Susceptibility Testing of *Salmonella* Isolates
372 from 2000 to 2007. J. Clin Microbiol. 47, 79–85. doi:10.1128/JCM.00894-08
- 373 8. CLSI. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for
374 Bacteria Isolated From Animals; Approved Standard – Fourth Edition. CLSI document
375 VET01-A4. Wayne, PA: Clinical and laboratory Standards Institute; 2013.
- 376 9. ISO/IEC 17025:2005, General requirements for the competence of testing and calibration
377 laboratories.

378

379

380 **Figure Legends**

381 Figure 1: Map indicating the participating countries colored with respect to the region they belong
382 to (Africa, Asia/Middle East, Caribbean, Europe, Latin America, North America, Oceania, Russia
383 or Southeast Asia). Performance with regard to the species identification and antimicrobial
384 susceptibility testing is indicated as average of correct results (%).

385 The 96 participating countries included the following: Africa (Algeria, Botswana, Cameroon,
386 Central African Republic, Congo Rep. of, Egypt, Ethiopia, Gambia, Gabon, Ivory Coast, Kenya,
387 Madagascar, Malawi, Mauritius, Morocco, Senegal, South Africa, Sudan, Tunisia); Asia & Middle
388 East (China, Iran Islamic Rep. of, Israel, Kuwait, Oman, Saudi Arabia); Caribbean (Barbados,
389 Grenada, Jamaica, Trinidad and Tobago); Europe (Bosnia and Herzegovina, Bulgaria, Croatia,
390 Cyprus, Czech Republic, Denmark, Estonia, Finland, Germany, Greece, Hungary, Iceland, Italy,
391 Latvia, Lithuania, Luxembourg, Macedonia, Malta, Rep. of Moldova, Netherlands, Norway,
392 Poland, Romania, Serbia, Slovakia, Slovenia, Spain, Turkey); Latin America (Argentina, Bolivia,
393 Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, El Salvador, Guatemala, Mexico, Panama,
394 Paraguay, Peru, Suriname, Uruguay, Venezuela); North America (Canada, United States of
395 America); Oceania (Australia, New Caledonia, New Zealand); Russian region (Belarus, Georgia,
396 Russian Federation, Ukraine); Southeast Asia (Brunei Darussalam, Cambodia, India, Japan, Korea
397 Rep. of, Lao Dem. Rep. of, Malaysia, Philippines, Singapore, Sri Lanka, Taiwan, Thailand, Viet
398 Nam).

399 Figure 2: Species identification of *Campylobacter*; summary of the performance per year of results
400 covering all nine participating regions (Africa, Asia/Middle East, Caribbean, Europe, Latin
401 America, North America, Oceania, Russia, Southeast Asia).

402

403 Figure 3: Species identification of *Campylobacter coli* and *C. jejuni*; summary of the performance
404 per region of results over the years 2003-2012 (excl. 2005).

405

406 Figure 4: Antimicrobial susceptibility testing of *Campylobacter*; the performance per year and
407 region.

408

409 Figure 5: Antimicrobial susceptibility testing of *Campylobacter*; summary of the performance per
410 year of results covering all nine participating regions (Africa, Asia/Middle East, Caribbean, Europe,
411 Latin America, North America, Oceania, Russia, Southeast Asia).

Figures for

“Proficiency of WHO Global Foodborne Infections Network External Quality Assurance System participants in the identification and susceptibility testing of thermo-tolerant *Campylobacter* spp. from 2003-2012”

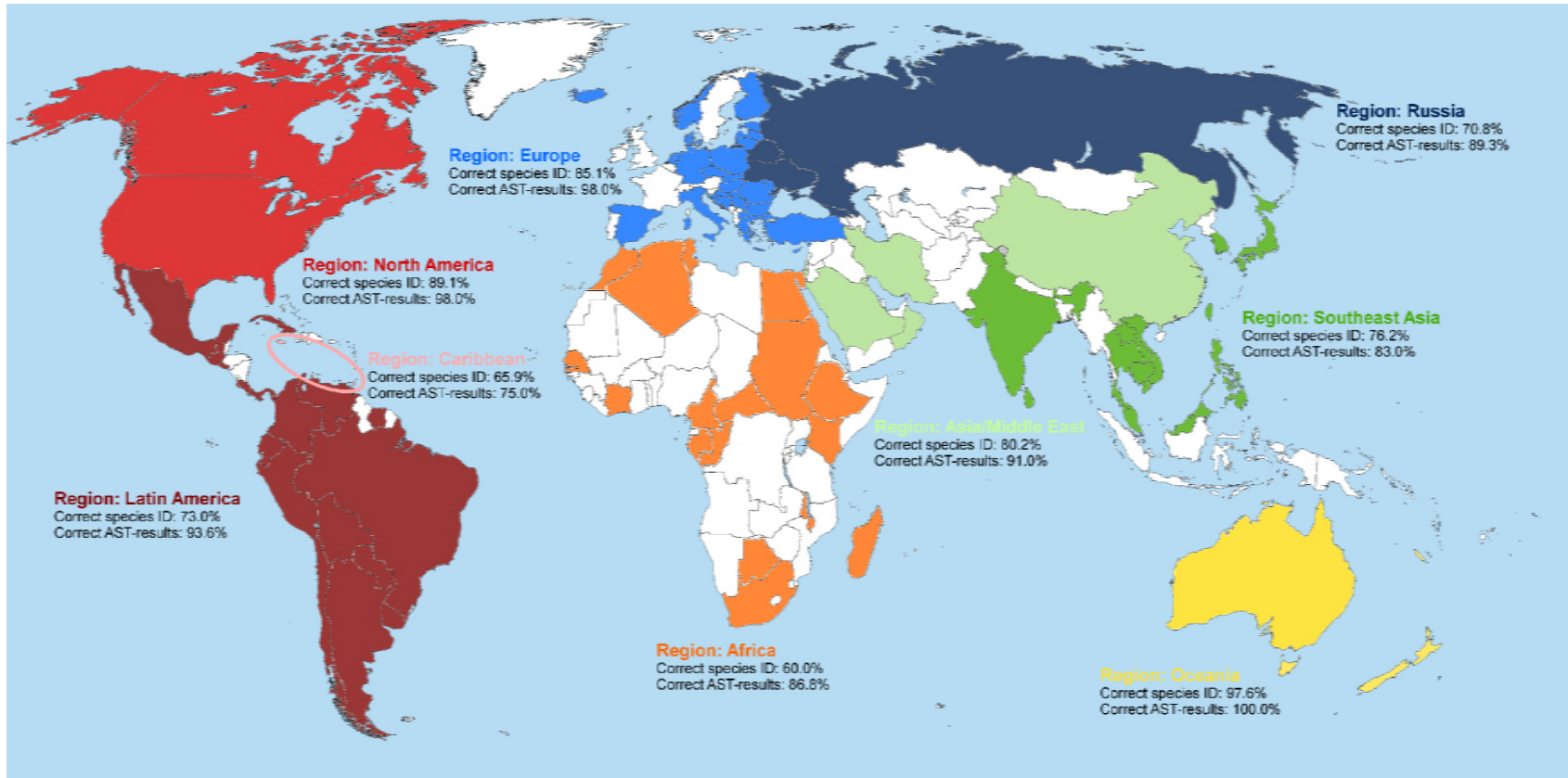


Figure 1: Map indicating the participating countries colored with respect to the region they belong to (Africa, Asia/Middle East, Caribbean, Europe, Latin America, North America, Oceania, Russia or Southeast Asia). Performance with regard to the species identification and antimicrobial susceptibility testing is indicated as average of correct results (%).

The 96 participating countries included the following: Africa (Algeria, Botswana, Cameroon, Central African Republic, Congo Rep. of, Egypt, Ethiopia, Gambia, Gabon, Ivory Coast, Kenya, Madagascar, Malawi, Mauritius, Morocco, Senegal, South Africa, Sudan, Tunisia);

Asia & Middle East (China, Iran Islamic Rep. of, Israel, Kuwait, Oman, Saudi Arabia); Caribbean (Barbados, Grenada, Jamaica, Trinidad and Tobago); Europe (Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Germany, Greece, Hungary, Iceland, Italy, Latvia, Lithuania, Luxembourg, Macedonia, Malta, Rep. of Moldova, Netherlands, Norway, Poland, Romania, Serbia, Slovakia, Slovenia, Spain, Turkey); Latin America (Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Ecuador, El Salvador, Guatemala, Mexico, Panama, Paraguay, Peru, Suriname, Uruguay, Venezuela); North America (Canada, United States of America); Oceania (Australia, New Caledonia, New Zealand); Russian region (Belarus, Georgia, Russian Federation, Ukraine); Southeast Asia (Brunei Darussalam, Cambodia, India, Japan, Korea Rep. of, Lao Dem. Rep. of, Malaysia, Philippines, Singapore, Sri Lanka, Taiwan, Thailand, Viet Nam).

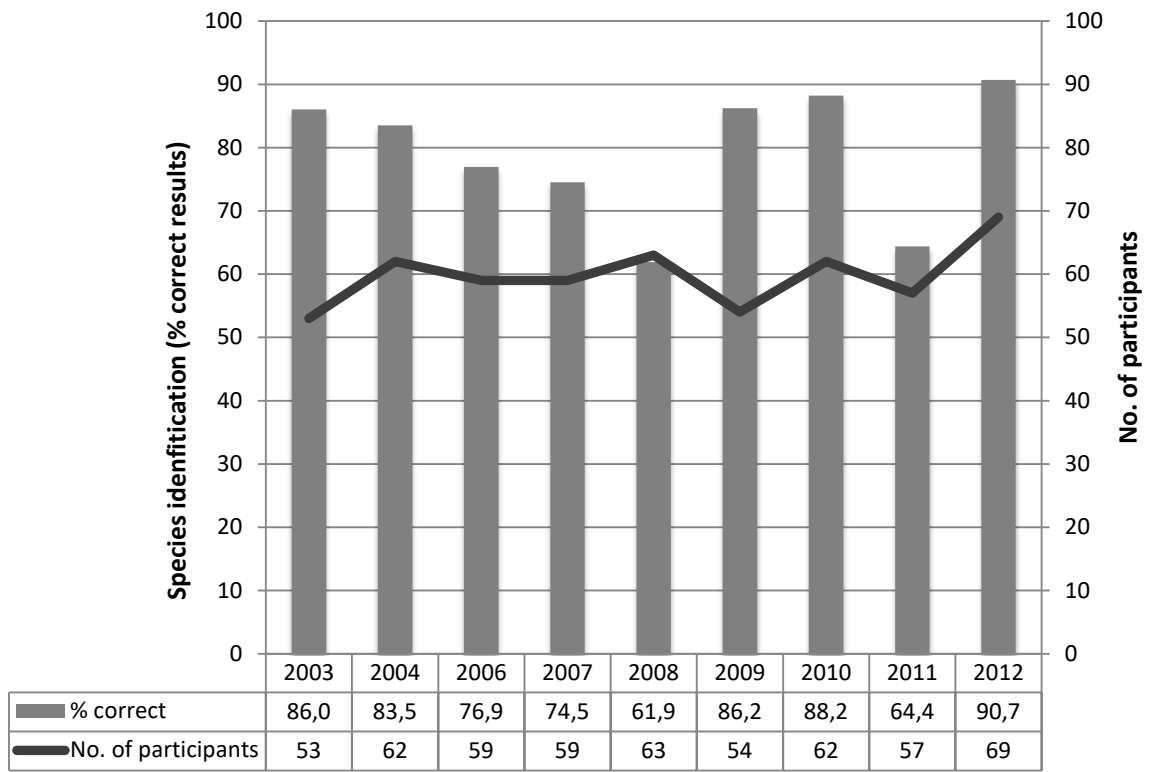


Figure 2: Species identification of *Campylobacter*; summary of the performance per year of results covering all nine participating regions (Africa, Asia/Middle East, Caribbean, Europe, Latin America, North America, Oceania, Russia, Southeast Asia).

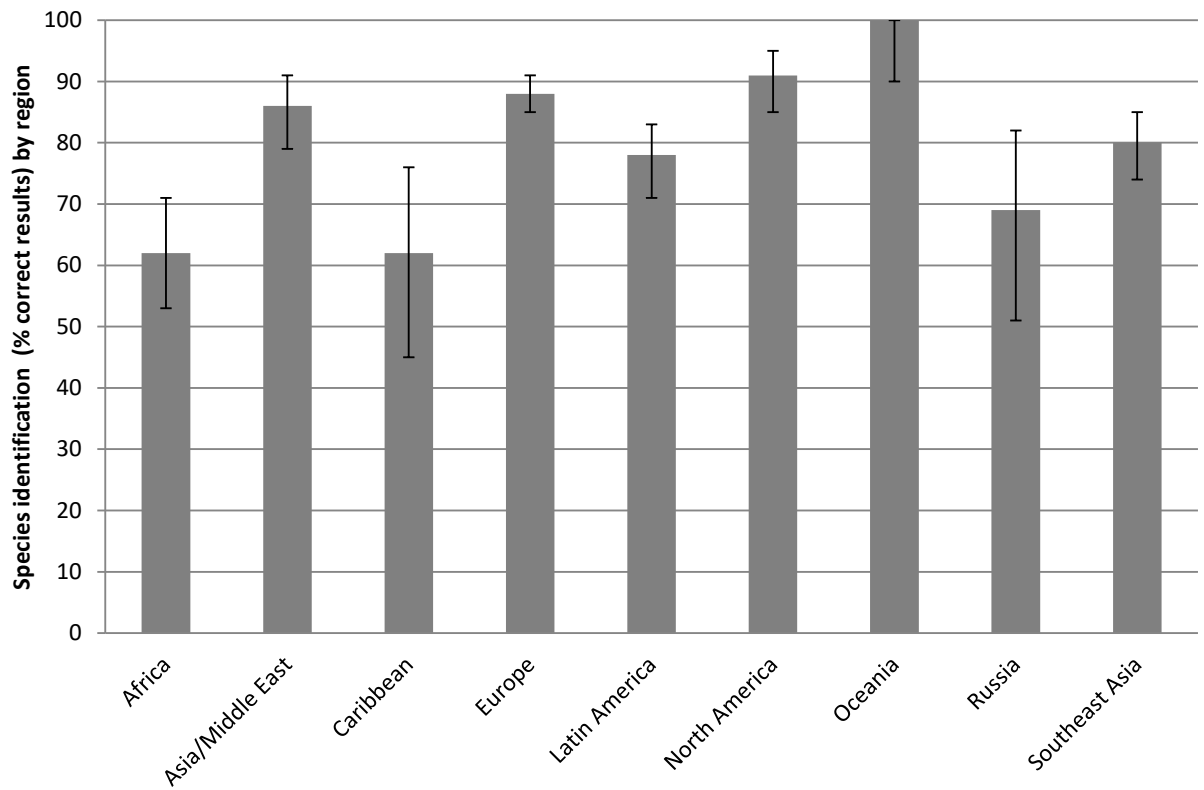


Figure 3: Species identification of *Campylobacter coli* and *C. jejuni*; summary of the performance per region of results over the years 2003-2012 (excl. 2005).

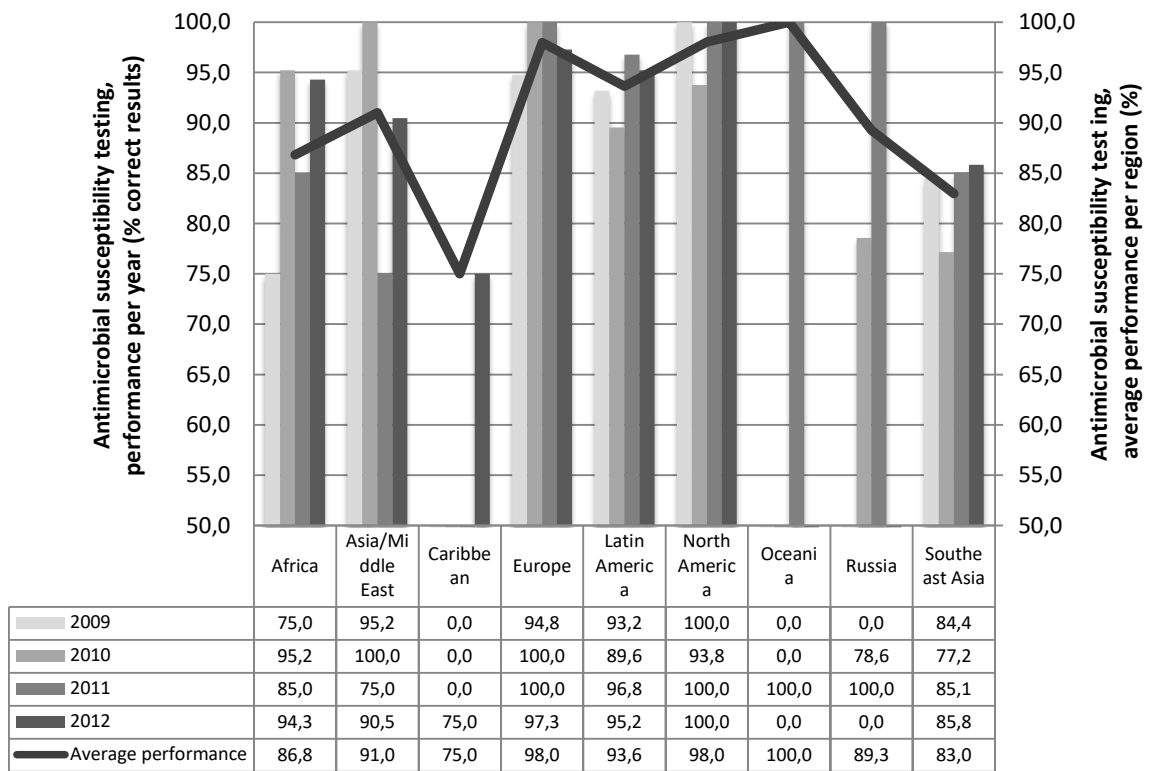


Figure 4: Antimicrobial susceptibility testing of *Campylobacter*; the performance per year and region.

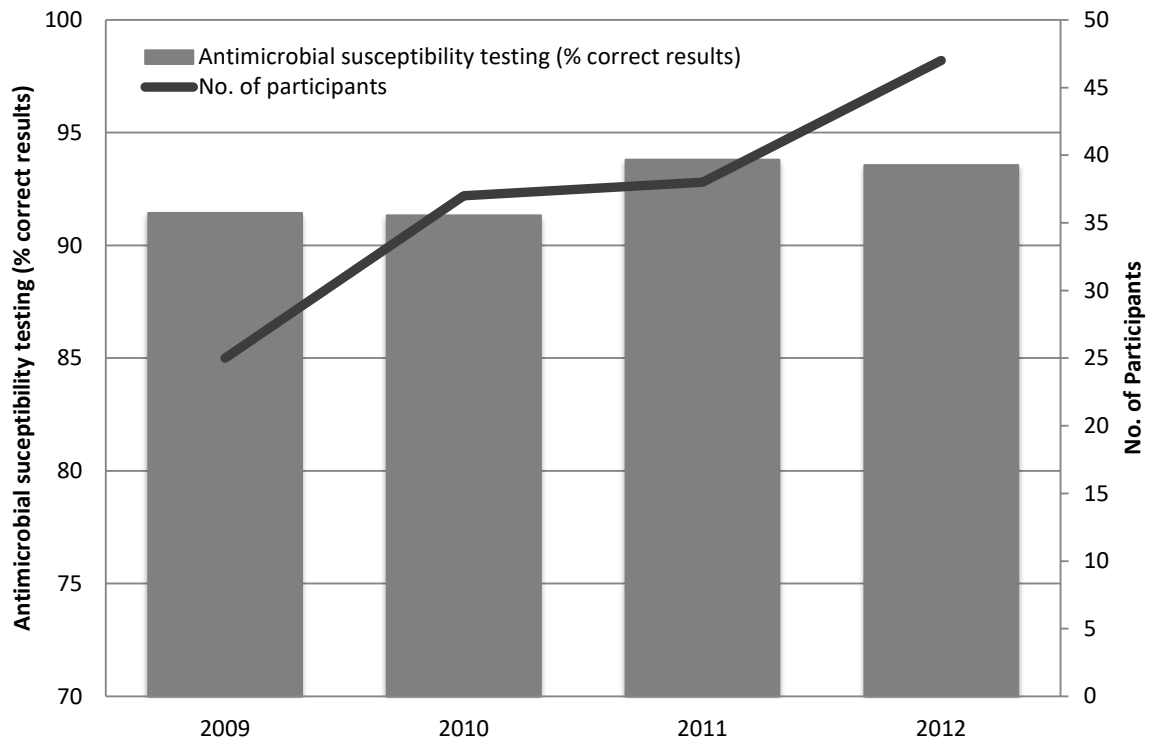


Figure 5: Antimicrobial susceptibility testing of *Campylobacter*; summary of the performance per year of results covering all nine participating regions (Africa, Asia/Middle East, Caribbean, Europe, Latin America, North America, Oceania, Russia, Southeast Asia).