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1 **Robotic Antimicrobial Susceptibility Platform (RASP): A Next Generation Approach to One-Health**
2 **Surveillance of Antimicrobial Resistance.**

3 **Running Title: Robotics for Antimicrobial Resistance Surveillance**

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6

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18 **Abstract**

19 *Background*

20 Surveillance of antimicrobial resistance (AMR) is critical to reducing its wide-reaching impact. Its
21 reliance on sample size invites solutions to longstanding constraints regarding scalability. A robotic
22 platform (RASP) was developed for high-throughput AMR surveillance in accordance with
23 internationally recognised standards (CLSI and ISO 20776-1:2019) and validated through a series of
24 experiments.

25 *Methods*

26 Experiment A compared RASP's ability to achieve consistent MICs to that of a human technician across
27 eight replicates for four *E. coli* isolates. Experiment B assessed RASP's agreement with human
28 performed MICs across 91 *E. coli* isolates with a diverse range of AMR profiles. Additionally, to
29 demonstrate its real-world applicability, the RASP workflow was then applied to five faecal samples
30 where a minimum of 47 *E. coli* per animal (239 total) were evaluated using an AMR indexing
31 framework.

32 *Results*

33 For each drug-rater-isolate combination in experiment A, there was a clear consensus of the MIC and
34 deviation from the consensus remained within one doubling-dilution (the exception being gentamicin
35 at two dilutions). Experiment B revealed a concordance correlation coefficient of 0.9670 (95%CI:
36 0.9670 - 0.9670) between the robot and human performed MICs. RASP's application to the five faecal
37 samples highlighted the intra-animal diversity of gut commensal *E. coli,* identifying between five and
38 nine unique isolate AMR phenotypes per sample.

39 *Conclusions*

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40 While adhering to internationally accepted guidelines, RASP was superior in throughput, cost and data
41 resolution when compared to an experienced human technician. Integration of robotics platforms in
42 the microbiology laboratory is a necessary advancement for future One-Health AMR endeavours.

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43 **Introduction**

44 Antimicrobial resistance (AMR) has been described as one of the greatest threats to global health and
45 food security, with an estimated cumulative cost of 100 trillion USD by 2050.[1] The major consequence
46 of resistance is the escalating difficulty of successfully treating bacterial infections with the currently
47 available limited array of antimicrobials. We now face the risk of returning to the pre-antibiotic era
48 where bacterial infections in humans and animals were a major cause of mortality.[2] Preserving the
49 usefulness of existing antimicrobials by reducing selection and limiting the dissemination of resistant
50 organisms is therefore a high priority. Consequently, a key component of management efforts is
51 surveillance designed to keep authorities and clinicians aware of where and when resistance is present
52 and evolving.[3] Surveillance of AMR faces many challenges owing to the multi-host, multi-pathogen,
53 multi-drug nature of the resistance phenomenon. Perhaps one of the most problematic aspects is the
54 insidious nature of resistance - it emerges slowly without necessarily yielding expression of any
55 outward signs and frequently has an impact at a place and time other than its origin. For surveillance
56 to overcome these obstacles, careful attention needs to be paid to the choice of pairing of assays for
57 measuring resistance in bacteria and the design of sample acquisition from animals, food and the
58 environment.

59 Surveillance for AMR in animals and food is a well-established activity. The WHO has longstanding
60 recommendations for the conduct of "integrated surveillance", as part of the multifaceted-
61 management for the control of resistance.[4] Countries such as Denmark[5] and the USA[6] have been at
62 the forefront of surveillance for AMR in animals and the food chain with programs running since as
63 early as 1995. Since then, the basic approach in food-animals, which is now widely adopted
64 throughout the developed world, has changed very little. A core component is the collection and
65 analysis of data relating to the AMR profile of indicator bacteria (such as *Escherichia coli*) from various
66 livestock and commodities.[7] As a result of the high cost per isolate (of which laboratory processing
67 contributes a significant portion), most national-level surveys typically only collect data on

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68 approximately 150-250 individually selected isolates per year from each livestock sector. While the
69 resulting data provide a general overview on AMR in food-animals, the inferences that can be drawn
70 are limited. In addition, recent data have demonstrated variation of *E. coli* resistance profiles within
71 bacterial species from the same host,[8] suggesting that reliable estimates of AMR require the collection
72 of multiple isolates from a single host.

73 In order to effectively limit the emergence and spread of AMR within groupings of animals or humans,
74 surveillance data must be strengthened to make it more relevant to antimicrobial stewardship.[9] A
75 paramount need is for the individuals responsible for antimicrobial stewardship in a setting to receive
76 intelligence from surveillance describing the occurrence of resistance. To deliver such feedback,
77 epidemiologically appropriate study design and sample size needs to be combined with cost-effective
78 characterisation of much larger numbers of bacteria than are currently evaluated.[10] At present the
79 isolation of large numbers of bacterial colonies, subsequent AST with traditional techniques and
80 genomic characterisation is not only cost-prohibitive but a severe drain on time and resources. Scaling
81 up traditional methods is unattractive because it would inevitably introduce inaccuracy arising from
82 fatigue of laboratory workers. However, the harnessing of high-throughput laboratory robotic
83 platforms capable of handling individual colonies on an unprecedented scale and without the loss of
84 accuracy from fatigue we associate with manual methods is a practical solution.

85 In this study, we describe the development of the next generation approach to surveillance for AMR
86 using a Robotic Antimicrobial Susceptibility Platform (RASP) and demonstrate that the RASP method
87 is elegantly and efficiently adapted to high-volume surveillance of commensal organisms. The system
88 automates the process of bacterial isolation, identification (with or without reliance on
89 spectrography), and AST for customised combinations of drugs. With this comes a much-needed
90 improvement in the ability to design surveillance to meet objectives that are of practical relevance at
91 the coalface of antimicrobial stewardship.

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92 **Methods**

93 **System overview**

94 RASP was developed to assess phenotypic resistance in a manner comparable with major surveillance
95 programs. Unlike other approaches to "scaling up" such as metagenomics, RASP keeps the nucleic acid
96 of individual isolates available for study making it possible to understand if resistance is mediated by
97 previously unidentified genes while avoiding biases due to the presence of extraneous nucleic acid
98 from environmental organisms.

99 RASP is a customisable robotics platform that was designed, using available Tecan (Switzerland) and
100 SciRobotics Ltd. (Israel) products, to be flexible and multifunctional, capable of bacterial isolation,
101 isolate collection, preparation for identification and AST. A Tecan Freedom EVO® 150 base was
102 combined with the SciRobotics carousel capable of holding both petri dishes and 96-well microplates.
103 SciRobotics equipment was deployed on the robot deck, the PetriPlater™ to dispense samples and
104 Pickolo™ software using image analysis to select colonies, in addition to a microplate absorbance
105 reader for AST inoculum adjustment to deliver high-throughput surveillance (Figure S1). The robot has
106 also been fully integrated with barcoding capabilities to track isolates from original source to
107 phenotypic results and is integrable with any electronic laboratory information management system.
108 The general workflow for this method is visualised in Figure 1 and the major steps detailed below.
109 Homogenised samples are loaded onto the robot in a liquid format and either serially diluted or
110 directly streaked onto agar. After incubation individual colonies are selected using Pickolo™, based on
111 the explicit requirements of the user including colour, size and morphology. Individual colonies are
112 transferred to a 96-well microplate and a deep-well plate in preparation for AST and isolate storage,
113 respectively. MALDI-TOF identification can also be performed at this timepoint and as such, the robot
114 includes a position for a MALDI-TOF target plate and is capable of both sample and matrix addition.
115 AST is then performed using an entirely liquid based methodology adapted for the RASP platform from
116 internationally established guidelines: Clinical Laboratory Standards Institute (CLSI)[11] and ISO 20776-

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117 1:2019; including compliance to quality control criteria. AST drug panels, genotyping and whole
118 genome sequencing can be prepared using the Freedom EVO genomics platform. While the above
119 workflow was well suited to this current study, it should be noted that it represents one of many
120 possibilities. RASP workflows are flexible and due to the modular design of hardware, can be adjusted
121 to suit different bacterial species, sample types, culture media and drug panels.

122 **Bacterial Isolation**

123 Two grams of faecal sample was homogenised in 18 mL PBS. Homogenised samples then underwent
124 four 10-fold serial dilutions and was plated onto CHROMagar™ ECC (Edwards, MM1076) using the
125 two-zone spiral plating protocol at 10^{-3} (outer zone) and 10^{-4} (inner zone) (Video S1). After overnight
126 incubation at 37°C, Pickolo™ colony picking software was used to target and pick up to 48 presumptive
127 *E. coli* colonies per plate based on their adherence to previously determined colour, size and circularity
128 criteria (Video S2) and transferred to 96-well plates containing CAMHB (BD, 212322) for overnight
129 growth at 37°C for storage and subsequent assays.

130 **Bacterial Identification**

131 Isolates were prepared for MALDI-TOF identification by adding 40 µL of 90% Formic acid to the
132 bacterial pellet from overnight culture. (Video S3). Species identification was then performed
133 by MALDI-TOF adhering to manufacturer's protocol (Bruker MALDI Biotyper Microflex LT/SH MALDI-
134 MS running MBT Compass 4.1 Build 70 and flexControl 3.4 Build 135).

135 **Antimicrobial Susceptibility Testing**

136 All isolates were grown from storage at -80°C onto sheep blood agar (Edwards, MM1120) overnight
137 at 37°C. A second subculture was performed the following day, and the workflow diverged depending
138 on the prospective rater.

139 *RASP Platform*

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140 The robot protocol adjusted isolates from the overnight broth culture using an absorbance reader at
141 620 nm wavelength, to an absorbance equivalent to a McFarland standard (0.08 to 0.13). The
142 McFarland standardised isolate was transferred to a deep well plate and diluted 1:20 in sterile
143 water, and the drug plate was inoculated with 10 µL of isolate in 90 µL of CAMHB diluted drug (Video
144 S4). For colony enumeration, the robot performed further dilutions (1:150 followed by another 1:150)
145 on the previously diluted McFarland standard and plated 100 µL on sheep blood agar using a
146 modification of the two-zone spiral dilution plating method; whereby the entire plate is inoculated as
147 just one zone (lawn plating). Following an overnight incubation (16 – 20 hours at 37°C as per CLSI
148 guidelines), drug plate results for both raters were read using the Sensititre Vizion plate reader
149 system, and colony enumeration on sheep blood agar was performed and recorded using
150 the Pickolo colony counting software.

151 *Human Technician*

152 Isolates intended for human performed susceptibility testing were subcultured on sheep blood agar,
153 while isolates intended for robot performed susceptibility testing were subcultured in a flat-bottom
154 96-well plate (Nunc, 167008), each well filled with 220 µL (allowing for overnight evaporation of
155 roughly 20 µL) CAMHB (BD, 212322). The human broth microdilution protocol followed CLSI
156 guidelines, with the adjustment of isolates to a 0.5 McFarland standard using a nephelometer
157 (Sensititre™) followed by a 1:100 dilution in CAMHB prior to inoculation of the drug plate
158 using Sensititre's AIM™ automated inoculation delivery system (50 µL inoculum into 50 µL of drug
159 diluted in CAMHB). The colony enumeration quality control step was performed for each isolate on
160 sheep blood agar as per CLSI guidelines with a 1:1000 dilution of inoculum in 0.9% saline and lawn
161 plating on agar.

162 **Validation and Application of RASP**

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163 To evaluate the capacity of the robot and validate its use in AST, we applied the RASP robotic platform
164 to the aforementioned workflow using a collection of *E. coli*; as *E. coli* is an ideal indicator organism
165 and represents a ubiquitous component of surveillance for AMR in animals.[5, 6, 12]

166 **AST Validation Experiment A: Assessment of Repeatability**

167 An experiment was conducted to validate the RASP platform's AST protocol by comparing the ability
168 of both human and robot rater to generate consistent minimum inhibitory concentrations (MICs)
169 across replicates. Each rater performed AST on four *E. coli* isolates in octuplicate against the following
170 antimicrobials: ampicillin, cefoxitin, ceftiofur, chloramphenicol, ciprofloxacin, colistin, ceftriaxone,
171 florfenicol, gentamicin, streptomycin, trimethoprim/sulfamethoxazole and tetracycline. Isolates were
172 derived from various sources including an American Type Culture Collection 25922, and one isolate of
173 seagull[13], porcine[14] and cattle origin[15], each with previously determined AMR profiles.

174 **AST Validation Experiment B: Assessment of Rater Agreeance**

175 Experiment B was a breadth study whereby the RASP platform and an experienced human technician
176 performed AST on a diverse range of isolates (n=91) with varying, previously characterised AMR
177 profiles and host origins (seagull, porcine, cattle).[13-17] The RASP platform was assessed based on its
178 agreeance with the human technician in their determination of MICs for each isolate. Antimicrobials
179 tested included: ampicillin, cefoxitin, ceftiofur, chloramphenicol, ciprofloxacin, colistin, ceftriaxone,
180 florfenicol, gentamicin, streptomycin, trimethoprim/sulfamethoxazole and tetracycline.

181 **RASP Workflow Application: Antimicrobial Resistance Surveillance Index Scoring**

182 To demonstrate the level of resolution achievable with RASP, five faecal samples were collected from
183 pig pen floors (one sample per pen) and proceeded through the RASP workflow described above to
184 yield AST data for 239 derivative *E. coli* isolates (minimum to 47 per sample) against six antimicrobials
185 (ampicillin, ceftiofur, ciprofloxacin, gentamicin, tetracycline and trimethoprim sulfamethoxazole). A
186 secondary classification and visualisation script devised in Stata[18] was applied to the 239 isolates to

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187 generate a graphical representation of intra-animal and intra-herd diversity, as well as to assign an
188 index score representative of the threat posed by their AMR burden. The indexing scheme assigned
189 weights (w) to antimicrobials informed by national[19] and international[20] guidelines whereby the
190 antimicrobials deemed more important to human health received a higher weighting. Antimicrobial
191 weights were as follows: ampicillin (w=1), ceftiofur (w=3), ciprofloxacin (w=4), gentamicin (w= 2),
192 tetracycline (w=1) and trimethoprim sulfamethoxazole (w=2). In this study the maximum possible
193 AMR score was 13, indicating resistance to all tested antimicrobials.

194 **Throughput Comparison Analysis**

195 An analytical component of this study sought to compare the time required by a human technician
196 utilising current methods, versus a robot-assisted technician, to process 20 homogenised faecal
197 samples (deriving 960 isolates) from isolation through to phenotypic characterisation. A simulated
198 workflow was developed for each technician based on average times taken to perform each task
199 within the workflow and applied to the above scenario. The human technician was allowed the use of
200 'modern' laboratory implements such as Sensititre's auto-inoculator and Vizion plate reader systems,
201 pre-ordered ready-to-use drug plates, and a MALDI-TOF, while the robot assisted technician had the
202 two previously discussed Tecan robots, a MALDI-TOF and a Sensititre Vizion plate reader system at
203 their disposal.

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204 **Results**

205 **AST Validation Experiment A: Assessment of Repeatability**

206 In Experiment A, when comparing the human (H) and robot (R) MIC results for each isolate, the mode
207 MIC of an isolate across both raters against a specific antimicrobial was labelled the 'consensus MIC'
208 and any deviation from the consensus MIC deemed a departure from the truest result (Figure 2). Of
209 the drug-rater-antimicrobial combinations tested (see Figure S2 for all combinations), the majority
210 show all eight replicates having achieved the same MIC result, and furthermore demonstrate
211 agreeance between the two raters; all isolates and replicates tested against tetracycline achieved the
212 same MIC between both isolate and rater. Of the cases where deviation from the consensus MIC was
213 seen, it was limited to one doubling-dilution (the exception being gentamicin at two dilutions) and
214 there remained moderate agreement between the two raters, with similar distributions of replicates
215 across MIC results.

216 **AST Validation Experiment B: Assessment of Rater Agreement**

217 In Experiment B, the majority of combinations of isolates and drugs (67.6 %) tested showed complete
218 agreement between the two human and robot raters while 23.4% had a discrepancy in MIC result by
219 one, which is acceptable according to international guidelines, and 9.0% had a discrepancy of two or
220 more doubling dilutions (Figure 3). The greatest discrepancy in MIC between raters was a difference
221 of nine dilutions between the two results and represented 0.26% of the isolate-drug combinations
222 tested. The overall agreement for isolate-drug combinations between robot and human was
223 summarised by a concordance correlation coefficient of 0.9670 (95%CI: 0.9670 - 0.9670), N: 1092.

224 **RASP Workflow Application: Antimicrobial Resistance Surveillance Index Scoring**

225 All homogenised faecal samples that were plated by RASP on CHROMagar™ ECC selective agars
226 yielded growth of at least 48 single colonies matching the expected blue appearance of *E. coli* at one
227 or both dilutions. Isolates were successfully identified as *E. coli* using RASP's MALDI-TOF preparation

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228 protocol, all with high identification scores; the lowest identification score achieved was 2.2 which
229 qualified within Bruker's highest confidence range.

230 Samples from all animals exhibited a diverse range of AMR profiles amongst isolates, including
231 resistance to antimicrobials tested (Figure 4). All animals, except one, had isolates representing an
232 AMR index of zero. The highest AMR index recorded was 13, comprising resistance to all tested
233 antimicrobials. A relatively balanced distribution of AMR indices was seen for the 48 isolates from
234 most animals; the exception being pig 'E', from which isolates were heavily skewed towards low AMR
235 indices, with a high density achieving an AMR index of one.

236 **Throughput Comparison Analysis**

237 It was estimated that the human technician would take 30 days to process these samples compared
238 to nine days for a robot-assisted technician (Figure S3). It's important to state that the robots were
239 not in continuous use during this workflow, meaning there was time where they could also have been
240 utilised for other tasks.

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241 **Discussion**

242 The impetus to transition microbiology from a manual-labour-centric profession to one embracing
243 automation is nothing novel. Automation offers the same advantage it has provided industry for
244 decades; the augmentation of processes to increase efficiency and throughput; and this statement
245 holds true for the application of the RASP platform to a conventional microbiology workflow. Typically,
246 several technicians would be required in the manual workflow depicted in Figure S3 to prevent the
247 occurrence of issues such as fatigue, and even still it is unlikely that optimal pace and quality of work
248 would be maintained for the entirety of the workflow; a problem to which the robot is immune. The
249 utilization of robotics instead allows staff to be diverted from monotonous and error-prone tasks to
250 more cognitively intensive ones such as data analysis and project management.[21, 22] These increases
251 to throughput are only significant, however, if the solution is financially viable. It is therefore
252 important to note that the improvement in processing efficiency is just one of several ways by which
253 automation alleviates sample processing costs; savings are also seen on materials and reagents as a
254 result of the transition to liquid-based methodologies (90% estimated cost reduction). In
255 consideration of the initial financial outlay of purchasing the RASP platform (400,000 AUD; equivalent
256 to approximately 300,000 USD or 260,000 EUR), we determined that approximately 9,000 samples
257 would need to be processed for the above savings to equate to this initial cost. The incorporation of
258 robotics into the routine microbiological assays described in this study is the technological leap
259 required to elevate the standard of future One-Health AMR surveillance.

260 While currently available laboratory robotic systems (e.g. VITEK®, Microscan, WASPLab® and BD
261 Phoenix™) do offer the automation of isolation, identification or antimicrobial susceptibility testing,
262 in comparison to RASP they utilise largely inflexible (in terms of pre-determined AST panels and
263 characterisation of a single isolate)[23] procedures to generate this information. We have developed the
264 first integrated system – from isolation to AMR profile production that meets international guidelines
265 and demonstrated that the method meets or exceeds the standards required. The Freedom EVO

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266 microbiology robot and by association the RASP protocol described here, utilises broth microdilution
267 testing and MALDI-TOF integration (both gold-standard microbiological procedures) to capitalise on
268 the higher throughput and resolution of data offered by its liquid-adapted methods.

269 AST completed by the robot was comparable to human generated data with similar patterns of MIC
270 variance observed. AST in its current format is highly variable as can be seen by the wide range of MIC
271 values acceptable for highly tested ATCC control strains.[11] This phenomenon was well depicted in
272 Experiment A, where under well-controlled, faithfully replicated conditions, MIC results from
273 replicates of the same isolate spanning two or three doubling dilutions were commonly observed. The
274 symmetry of MIC distributions between raters in these instances suggests that the source of this
275 variation is biological in nature, due perhaps, to phenomena such as isolates expelling their plasmid,
276 delayed expression of AMR genes or natural assay variations, as opposed to a technical failure by
277 either rater.

278 Due to the restrictions of conventional methods considered above, most surveys will collect
279 information for approximately 200 isolates per year per country, which limits the inferences that can
280 be drawn. For example, with respect to the ability to detect early-emergence of resistance to a key
281 drug, a simple application of binomial probability (expressed as: $P(X>0) \mid X \sim Bin(200, 0.01)$)
282 demonstrates that a survey relying on 200 isolates has a probability of only 13.4% of detecting any
283 positives if only 1% of isolates in the population have the resistance trait of interest. This calculation
284 substantially underestimates the true number of isolates required during surveillance because
285 reliance on a multi-staged sampling design (selection of herds, then animals, then isolates) is essential
286 and carries a cost in accuracy created by the design effect.[24] The AMR indexing experiment included
287 in this study supports other data[8, 25] in confirming that variation of *E. coli* resistance profiles within
288 bacterial species from the same host and same group of animals is commonplace. The high level of
289 resolution available from RASP for describing resistance in microbial populations, and its ability to
290 yield an affordable increase in the number of samples and isolates assessed, was demonstrated in the

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291 final validation experiment in this study by the diversity of AMR profiles. In this final study only 0.4%
292 (n=1) of tested isolates had an AMR index of 13, 1.7% (n=4) had resistance to gentamicin and 1.3%
293 (n=3) had resistance to ceftiofur. There was also a corresponding variation in the number of AMR
294 profiles for *E. coli* within a host: there being from 6-9 distinct variants detected in individuals, and
295 more would likely be detected with higher sample sizes. With the availability of RASP there is an
296 unprecedented opportunity to strengthen the management of AMR by shifting from a focus of
297 interpretation adapted to clinical isolates to one that expressly accommodates the diversity of
298 resistance in hosts at every level of organisation. Data generated on an expanded scale by RASP
299 therefore presents an opportunity to provide more meaningful guidance on antimicrobial stewardship
300 at the farm level through to national and international policy.

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301 **Conclusion**

302 RASP and technologies alike, unlock a higher calibre of AMR surveillance by overcoming longstanding
303 constraints to scalability. The flexibility of RASP permits its application well beyond the scope
304 demonstrated here, to the diverse range of bacterial landscapes encountered in the One-Health
305 system. This study saw RASP's workflow benchmarked against that of a contemporary laboratory,
306 where it was demonstrated to be equivalent to an experienced human technician but proved superior
307 in throughput, endurance and cost. It is critical that microbiology harnesses robotic platforms like
308 RASP if we are to resist the current trajectory of AMR.

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312 **Author Contributions**

313 A. T., R. A., Z. L., T. L. and S. A. designed and performed the plating and colony picking experiments. A.
314 T., R. A., J. B. and S. A. designed and performed the antimicrobial susceptibility testing experiments.
315 J. T. and D. T. provided consultation on the antimicrobial susceptibility testing experiments as well as
316 assisting with its validation according to international guidelines. S. K. developed the RASP platform
317 adapting the hardware and software for microbiological use and provided consultation throughout
318 the study. S. A., D. J., R. A., M. O. and D. T. conceived the project and S. A., R. A., M. O. and D. J.
319 supervised the project. A. T., R. A., D. J., T. Lee. and S. A. performed data analysis. A. T., R. A., D. J. and
320 S. A. wrote the initial draft of the manuscript, T. L., A. T., R. A. and S. A. designed figures and all authors
321 contributed to editing and proof reading of the manuscript.

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322 **Transparency Declaration**

323 The authors declare no competing interests.

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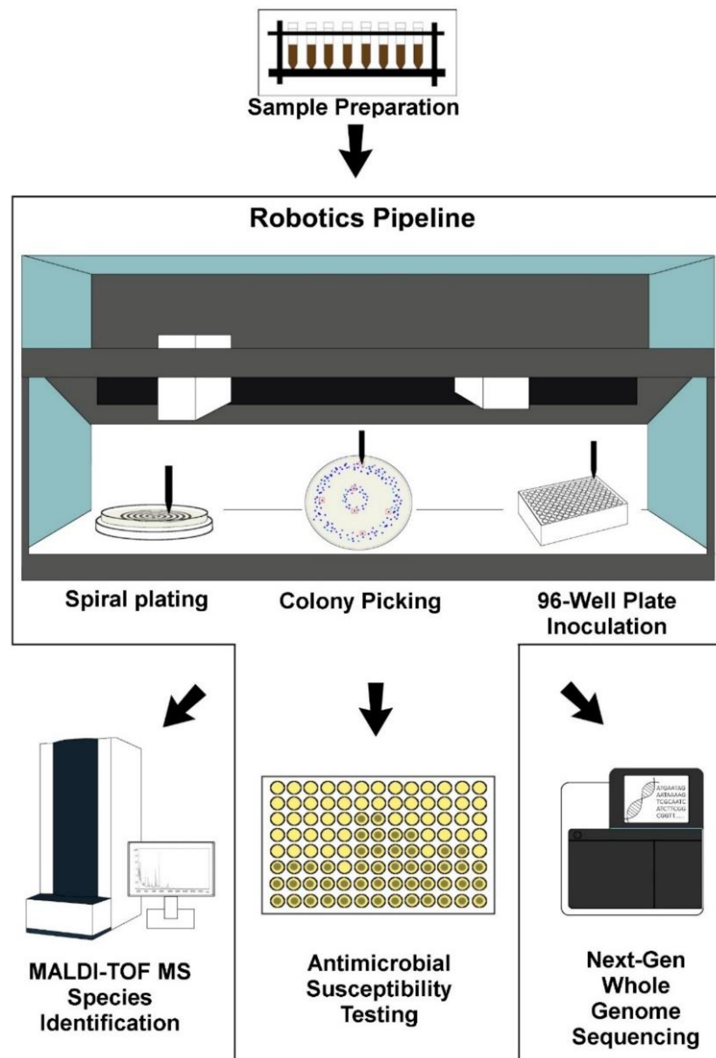
389 **Figures**



390

391 **Figure 1** | Overview of a typical RASP workflow. Any processes depicted outside the border occur externally to RASP.

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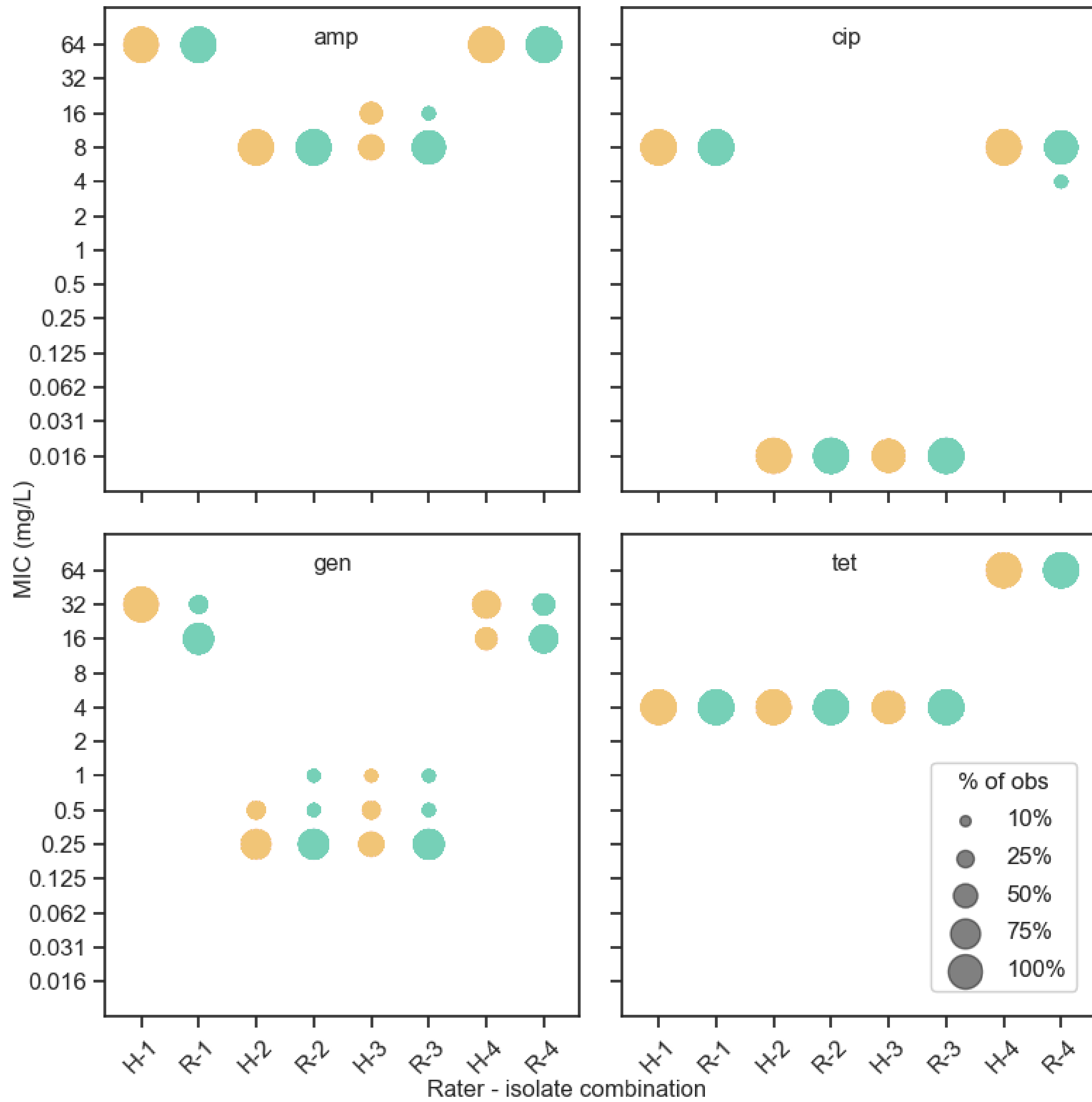

392

393 **Figure 2** | Experiment A: comparison of human ('H') and robot ('R') minimum inhibitory concentration (MIC) results of four
394 fully susceptible *E. coli* isolates each replicated eight times per rater, tested against a panel of antimicrobials. Each isolate-
395 rater designation displays the MIC results for all eight replicates of that combination. Antimicrobials: amp, ampicillin; cip,
396 ciprofloxacin; gen, gentamicin; tet, tetracycline.

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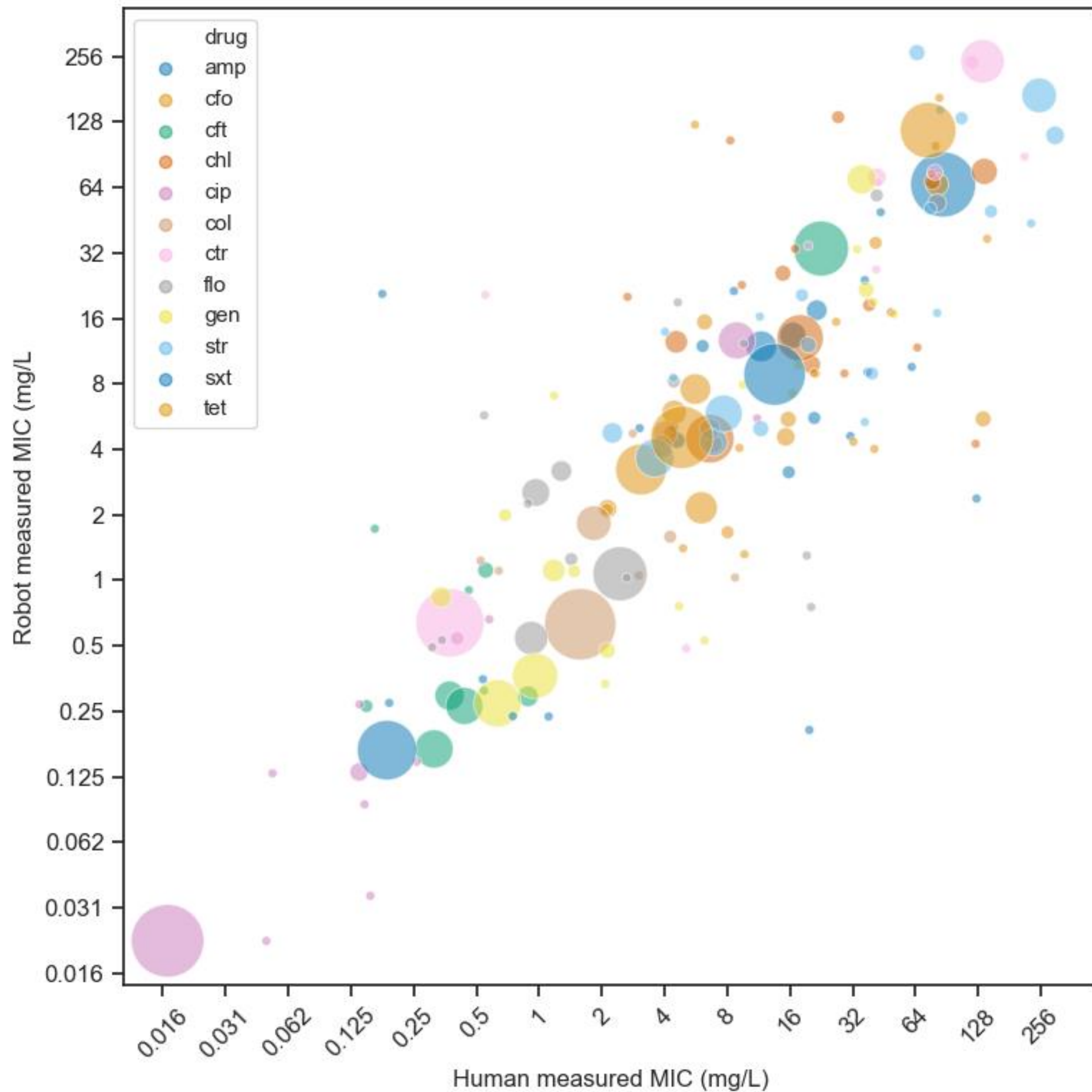

397

398 **Figure 3** | Experiment B: Paired minimum inhibitory concentration (MIC) results on 96 isolates with 12 drugs directly
399 comparing human and robot measurements, marker size represents proportion of paired observations for the isolate-drug
400 combination. Antimicrobials: amp, ampicillin; cfo, cefoxitin; cft, ceftiofur; chl, chloramphenicol; cip, ciprofloxacin; col,
401 colistin; ctr, ceftriaxone; flo, florfenicol; gen, gentamicin; str, streptomycin; sxt, trimethoprim/sulfamethoxazole; tet,
402 tetracycline.

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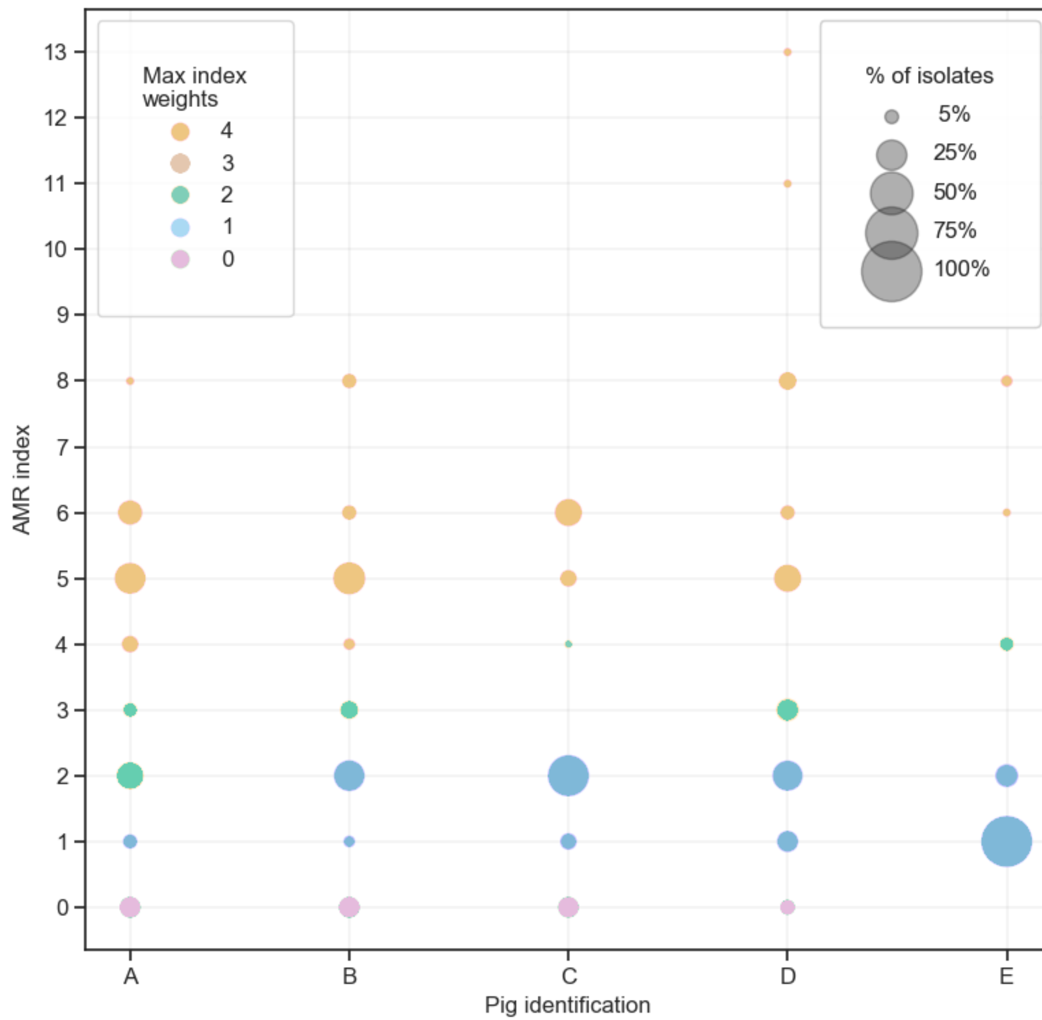

403

404 **Figure 4** | Antimicrobial resistance indexing scheme applied to 239 porcine *E. coli* isolates (48 from pigs A, B C and E, 47 from
405 pig D) using the following antimicrobial risk-weightings (w): ampicillin (w=1), ceftiofur (w=3), ciprofloxacin (w=4), gentamicin
406 (w= 2), tetracycline (w=1) and trimethoprim sulfamethoxazole (w=2). Colours are representative of the highest weighted
407 resistance present for an isolate.

408