Report on Farm-level Antimicrobial Usage and Resistance in Hong Kong SAR Food-producing Animals 2019

1. Background

Antimicrobial resistance (AMR) has been elevated to an issue of international concern by the United Nations. All countries/regions are expected to introduce measures to assist in containing this threat. Hong Kong Special Administrative Region (SAR) has been highly active in this area and, in 2017, released the Hong Kong Strategy and Action Plan on Antimicrobial Resistance 2017-2022 (the Action Plan).

The Action Plan required development of an appropriate surveillance programme for antimicrobial usage (AMU) and antimicrobial resistance (AMR) for local pig, chicken, and fish farms as part of the integrated, One Health monitoring and reporting system. According to the Action Plan this surveillance programme was expected to be operating in 2019.

A consultancy study was commissioned in 2017 to devise and recommend an appropriate surveillance programme. The system put in place was based on recommendations from the consultancy study conducted for AFCD from November 2017 to March 2019. The recommendations from this study were based on the unique characteristics of Hong Kong food animal production but also incorporated features of programmes from other countries. The surveillance programme, based on the recommendations of the consultancy study, officially commenced operation in mid-2019.

The programme was designed in conjunction with local food animal producers who saw the importance of addressing this issue and of providing information on AMU.

This report provides details of results from the first year of operation of the surveillance programme (2019) as well as some information from 2018 when preliminary data were collected on a trial-basis.

The data in this report provides a baseline against which changes over time can be assessed on local farms. AMR in livestock in Hong Kong SAR and the broader East and South East Asian region is already present, including resistance to highest priority critically important antimicrobials, such as 3rd and 4th generation cephalosporins and fluoroquinolones (see, for example, Cheng et al. 2015, Zhang et al. 2017). This is present even on farms that do not use these antimicrobials. It will likely take many years for improvements to be detected in levels of resistance even with well-managed antimicrobial (AM) stewardship programmes in place.

1.1 The Local Food Animal Production Industry

Food animals produced in Hong Kong represent only a small part of the animal-origin food consumed in Hong Kong, according to data derived or calculated from the Food and Environmental Hygiene Department and Census and Statistics Department. Local chicken farms currently produce all of the live birds sold in live-bird markets but this represents less than 3% of the total poultry consumed in Hong Kong. In 2018, local pigs represent about 6.7% of the live pigs sold and only 2% of the total pig products consumed. In 2019, because of the effects of the outbreak of African swine fever in the mainland, the contribution of local pigs increased to 15.6% of live pigs in the second half of the year and approximately 3% of total pig products. As of January 2019, there were 28 active and one inactive licensed chicken farms and 40 active and 3 inactive licensed pig farms.

In 2019, the total marine fish culture production (excluding shellfish) was equivalent to about 1 per cent of marine fish consumed (excluding fish fillet, meat, liver, roe and processed products such as dried, salted and smoked products) in Hong Kong. The total pond fish culture production (excluding shellfish) was equivalent to about 4% of freshwater fish consumed (excluding fish fillet, meat, liver, roe and processed products such as dried, salted and smoked products). There were 105 active and 817 semi-active/inactive licensed marine fish farms, and 248 active and 87 semi-active/inactive pond fish farms as of 31 Dec 2019.

As a result of the relatively small size of the production animal sector in Hong Kong it is unlikely to contribute much to the overall “resistance gene pool” in or on food of animal origin in Hong Kong. Nevertheless, local farms are expected to implement good disease prevention practices and adopt AM stewardship programmes as components of overall farm health management plans.

Funding has been approved under the Sustainable Agricultural Development Fund (SADF) and Sustainable Fisheries Development Fund (SFDF) for City University to provide veterinary services to pig, chicken and fish producers in Hong Kong SAR. The veterinary services for pig and chicken producers commenced in March 2019, and for fish producers in September 2018. As the systems develop and trust between City University and farms increases it is expected to result in improved disease control and prevention, better information on reasons for usage of antimicrobials, improved AM stewardship, reduced usage of highest priority, critically important antimicrobials and supply of AMs on prescription.

2. Antimicrobial Usage (AMU)

The AMU system adopted for local pig and chicken farms is based on a combination of reports from farmers of actual usage, usually provided on a monthly basis, and audit testing to detect additional usage that has not been reported or was not known by farmers (due, for example, to carry over of AMs between batches of feed or inclusion of AMs in feed that may not be present on labels). Since 2017, AFCD has been working with farmers to build this system by gathering information on the AM products that each farm is using.

This system differs from those used elsewhere that rely largely on data on sale of AMs. Comprehensive sales data are not available in Hong Kong for farmed food animals because of the limited availability of drugs locally. This problem is expected to be overcome once City University provides a full range of veterinary services to food animal producers, including supply of AMs. This means that the only way to obtain information on AMU, at present (until AMs are only available on prescription, including AMs for use in feed), is through voluntary reports from farmers, backed by audit testing. Data collected at the farm level can provide a much more accurate and granular picture of AMU than sales data. Most countries are moving towards collection of information at the farm level.

There are various ways to analyse and present AMU data. As recommended by the consultancy study, the data can be reported using four different metrics for pigs and chickens and two for aquaculture. A table providing information on the metrics used, their strengths and weaknesses of the metrics and reasons for their adoption is provided in the Methods section of the report in Annex 2.

A summary of the main findings is provided below. Details of methods used for collecting data are provided in Annex 2.

Main findings on AM usage

Very limited usage of antimicrobials in chicken and fish production with >80% of chicken farms reporting no known usage of AMs

Consistent with findings in other countries, the pig industry uses higher levels of AMs than chicken producers

No apparent increase in total usage in pigs occurred in 2019 compared to 2018 despite a small increase in pig production in 2019 (due to reduced supply of pigs from the mainland)

No known usage of the WHO highest priority, critically important AMs, colistin, fluoroquinolones and 3rd/4th generation cephalosporins in fish production

No known usage of colistin or fluoroquinolones and extremely limited usage (one farm) of 4th generation cephalosporins in chicken production (1.2 kg)

Limited usage of highest priority critically important AMs in pigs and only for treatment of disease, mainly by injection (total of 7.66 kg of fluoroquinolone, 12.12 kg of 3rd generation cephalosporin, 0.83 kg of colistin)

Testing of feed also allowed detection of carryover of low levels of AMs which, in some cases, may be present in purchased feed

Because of the low numbers of farms, changes in usage on one farm can have disproportionate effect on overall usage, especially for chicken farms

The highest quantities of AMs were provided via feed with only limited use of water medication (in part due to absence of suitable systems for delivering AMs via water)

Information was provided to higher level users on options for reducing usage of AMs

Farmers reported no known use of AMs for growth promotion, a stance that the industries support

Audit testing detected cases of inclusion of kitasamycin in feed given to chickens, unknown to the farmers, and several cases of inclusion of virginiamycin

2.1 Reported Usage

This section provides information on the quantities of AM used, as reported by farmers, broken down by species.

2.1.2 Reported usage of antimicrobials on chicken farms

Of the 29 licensed chicken farms, one is an inactive farm and was excluded from tables and graphs (the licensee reported 0 usage in 2019). Two farms are also excluded from the results below as they did not submit any AMU reports in 2019[1]. Therefore, a total of 26 farms are included in the AMU (mg/kg TAB) graphs below for the chicken industry in 2019.

Table 2.1 provides a summary of reporting by chicken farmers in the second half of 2018 and all of 2019. For details on calculations see Annex 2.

Table 2.1 Summary of chicken sector AMU reporting using different metrics

	2018*	2019
Monthly average AMU reporting rate	78%	84%
Number of farmers reporting AMU at least once in the year	27/29	27/29
Calculated total quantity of AMU in kg	65.46	143.57
AMU in mg/kg TAB	9.56	20.62
AMU in mg/kg PCU	16.93	36.56
AMU in DDDvet/1000 animal-days at risk	8.18	16.51

*Data was collected on a trial basis in the last 7 months of 2018. Note one farm who was the largest user in 2019 did not provide any reports in 2018. If this farm was not included the calculated total AM usage in 2019 was 64.68 kg. The apparent increase between 2018 and 2019 was due to inclusion of this farm in 2019.

Figures 2.1 and 2.2 provide a breakdown of total usage as reported by chicken farmers by quantity used.

[1] Audit testing was conducted on these farms via feed testing and in one case faecal waste testing.

Figure 2.1 Total AMU reported by chicken farmers in 2019 by AM class (kg)

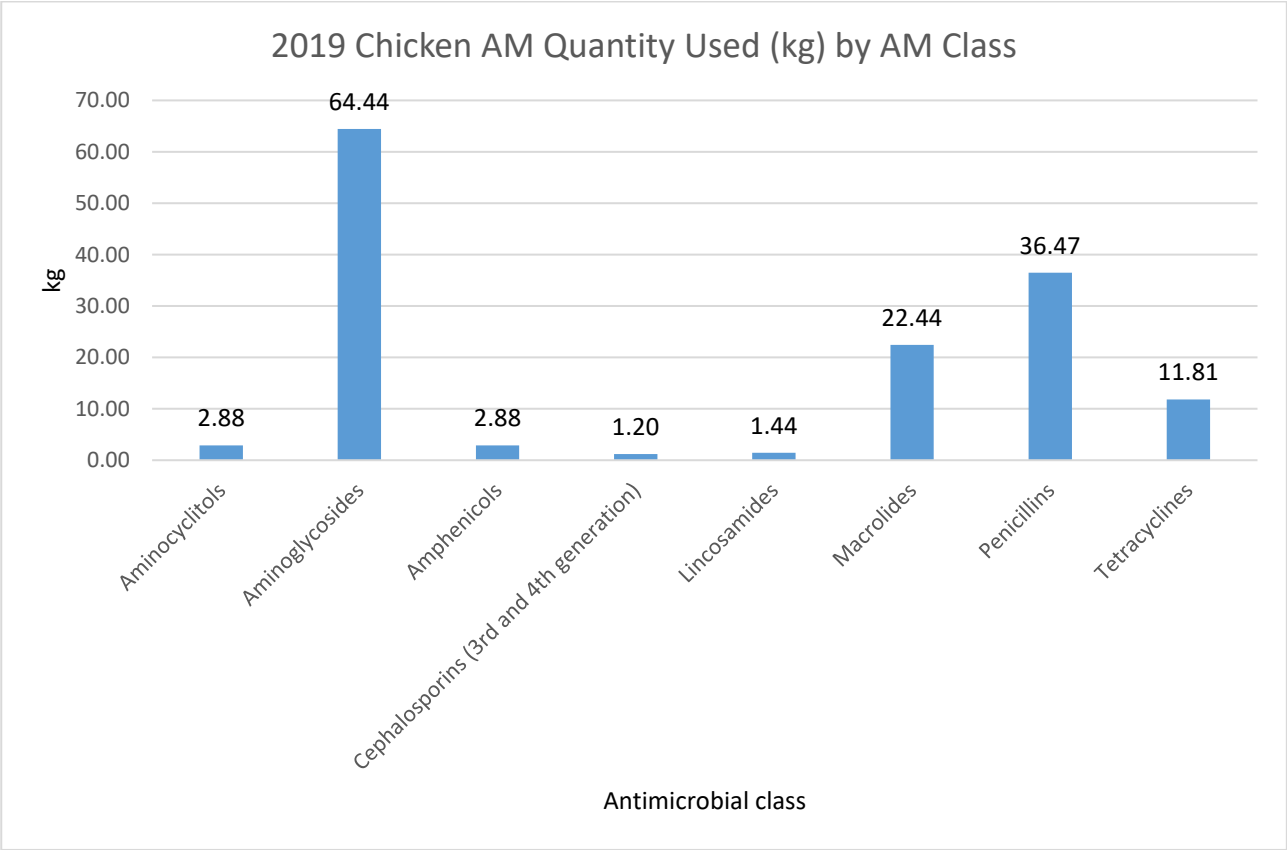


Figure 2.2. Total AMU reported by chicken farmers in 2019 by AM class (%)

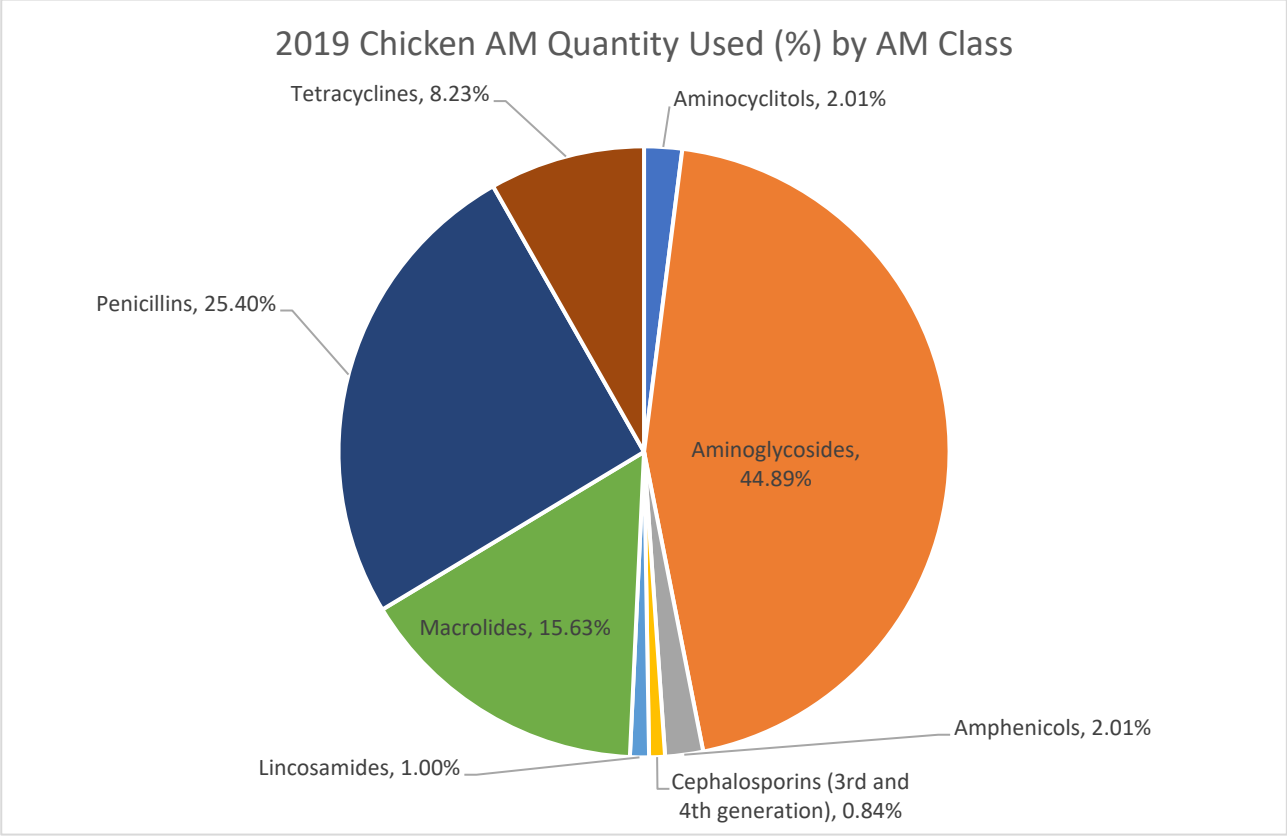


Table 2.2 provides a breakdown of reported farm usage of AMs for chicken farms. The main AM class used by quantity was aminoglycosides.

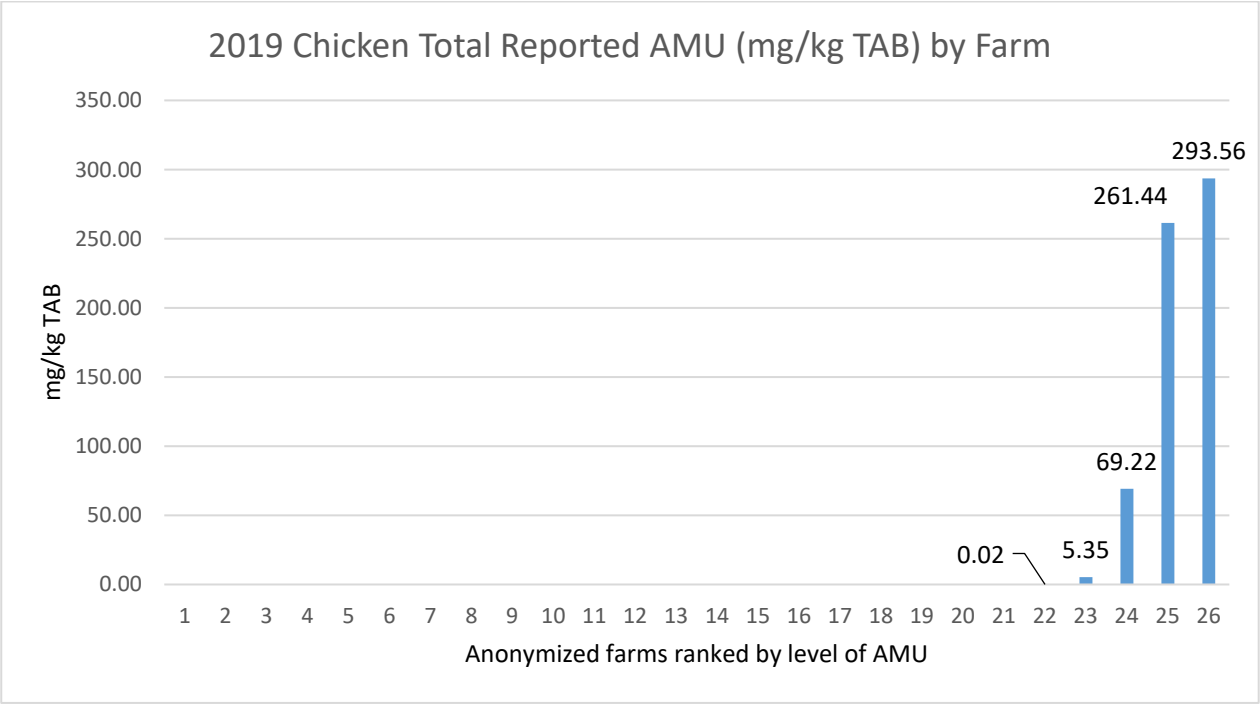
Limited quantities of the 4th generation cephalosporin, cefquinome, were used and only on one farm.

Table 2.2 Breakdown of AMU by number of chicken farms reporting usage, range of quantities used and AMs used

Antimicrobial class	Farms reporting usage	Range of usage (mg/kg TAB) excluding zero usage	AMs used
Aminocyclitols	2/26	1.8-19.8	Spectinomycin
Aminoglycosides	2/26	35.0-264.4	Amikacin, Neomycin
Amphenicols	1/26	10.7	Florfenicol
Cephalosporins (3rd and 4th gen)	1/26	4.5	Cefquinome
Lincosamides	2/26	0.9- 9.9	Lincomycin
Macrolides	2/26	39.6-65.6	Tylosin
Penicillins	2/26	0.02-135.7	Amoxicillin, Ampicillin
Tetracyclines	2/26	5.4-39.4	Doxycycline, Tetracycline

Data provided by chicken farmers indicated that five farms were knowingly providing their birds with antimicrobials (Figure 2.3). Two of these farms accounted for over 90% of total reported usage. Once results from feed testing (see audit testing) were also included another 9 farms were found to have antimicrobials in feed at levels typical of those used for treatment of disease (kitasamycin (5 farms), virginiamycin (3 farms) and oxytetracycline (1 farm)) although farmers were generally not aware that AMs were present in their feed.

Figure 2.3 Total reported AMU by chicken farm (mg/kg TAB)



The following two graphs (Figures 2.4 and 2.5) provide information on the quantities of AM used by class and includes information collected during 2018. Note that the apparent increase between 2018 and 2019 was due to the inclusion of one farm that did not report usage in 2018.

Figure 2.4 AMU by mg/kg TAB in chicken production in 2018 and 2019

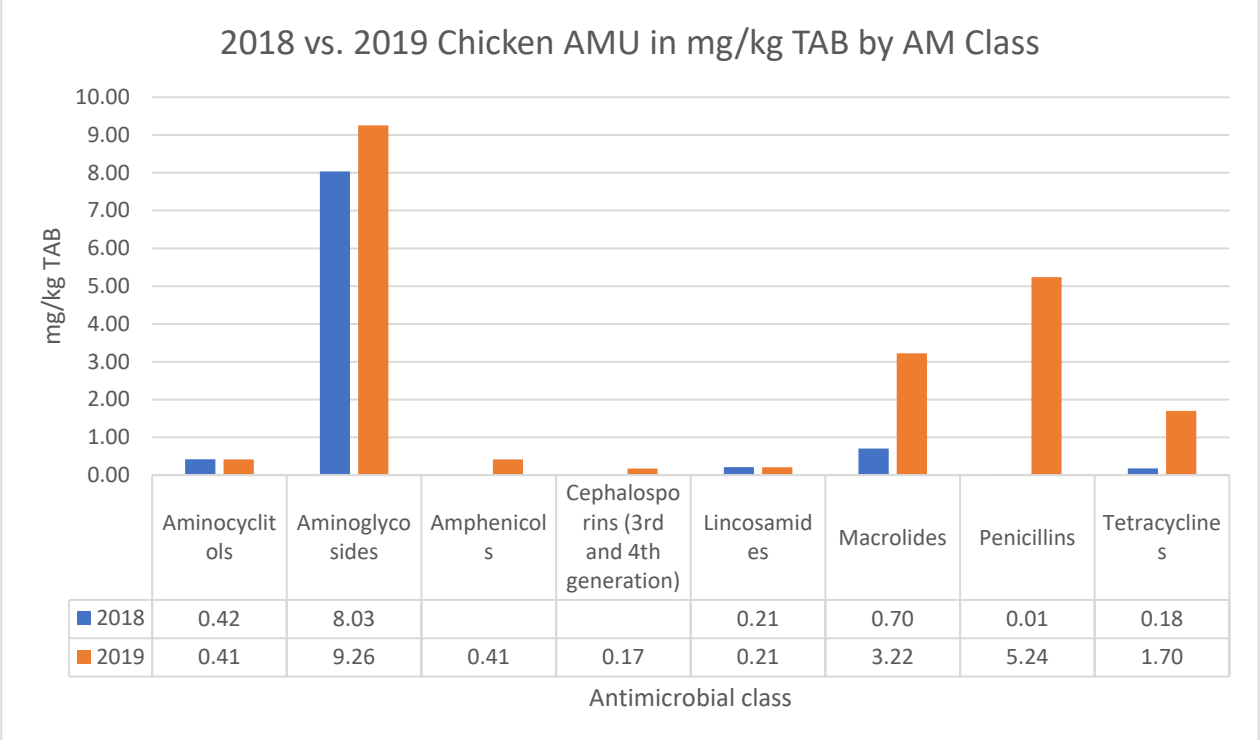
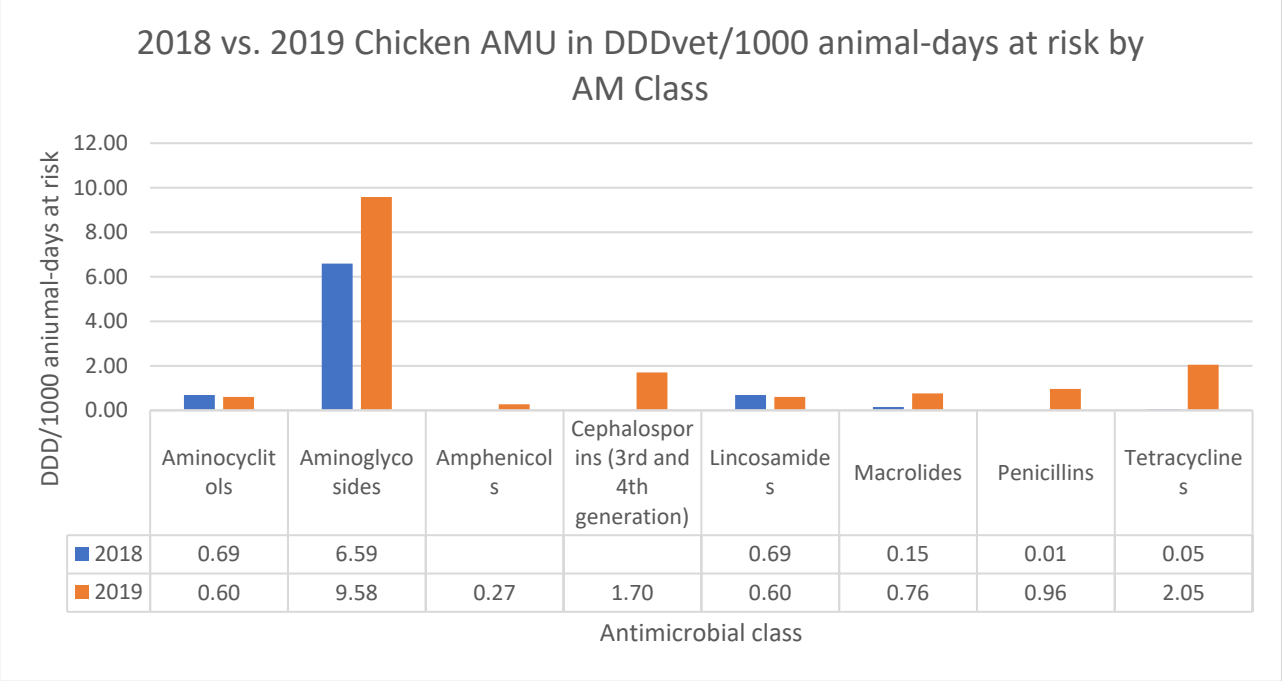


Figure 2.5 AMU by DDDvet/1000 animal-days at risk in chicken production in 2018 and 2019



2.1.2 Reported usage of antimicrobials on pig farms

Table 2.3 provides a summary of reporting by pig farmers in the second half of 2018 and all of 2019. For details on calculations see Annex 2.

Table 2.3 Summary of pig sector AMU reporting using different metrics

	2018	2019
Monthly average AMU reporting rate	75%	84%
Number of farmers reporting AMU at least once in the year	41/43	41/43
Calculated total quantity of AM used in kg	1742.68	1753.49
AMU in mg/kg TAB	114.88	111.18
AMU in mg/kg PCU	204.53	194.97
AMU in DDDvet/1000 animal-days at risk	66.85	61.43

Figures 2.6 and 2.7 show the total quantities used in kg and by proportion by AM classes. By quantity, the most used AMs are penicillins (especially amoxicillin), tetracyclines (chlortetracycline, oxytetracycline, doxycycline), amphenicols (florfenicol) and macrolides whereas by DDD amphenicols (florfenicol) are used more commonly.

The main macrolides used are tylosin and tilmicosin which are used mainly for treatment of respiratory disease complex in pigs.

Figure 2.6 Total AMU reported by pig farmers in 2019 by AM class (kg)

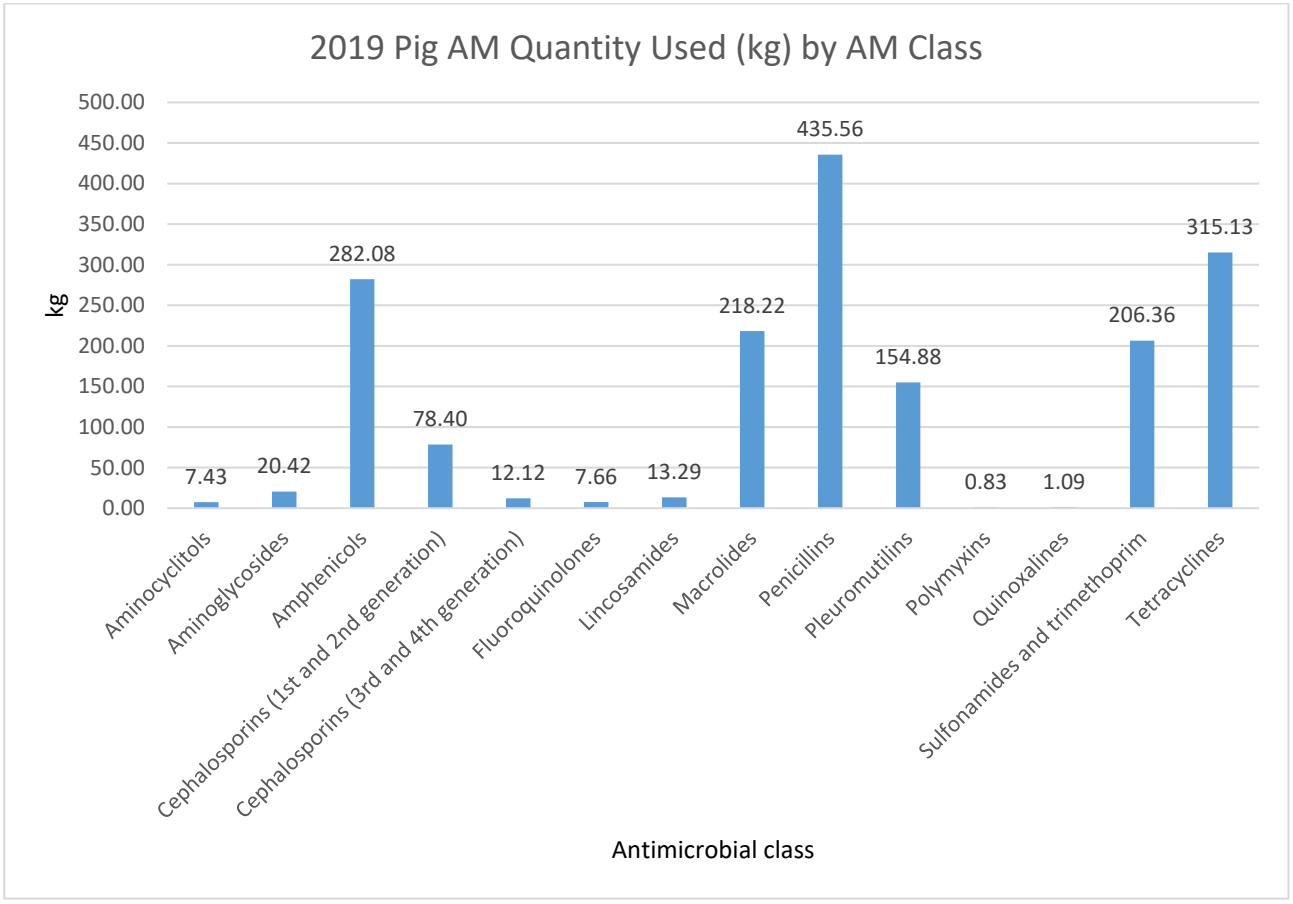


Figure 2.7 Total AMU reported by pig farmers in 2019 by AM class (%)

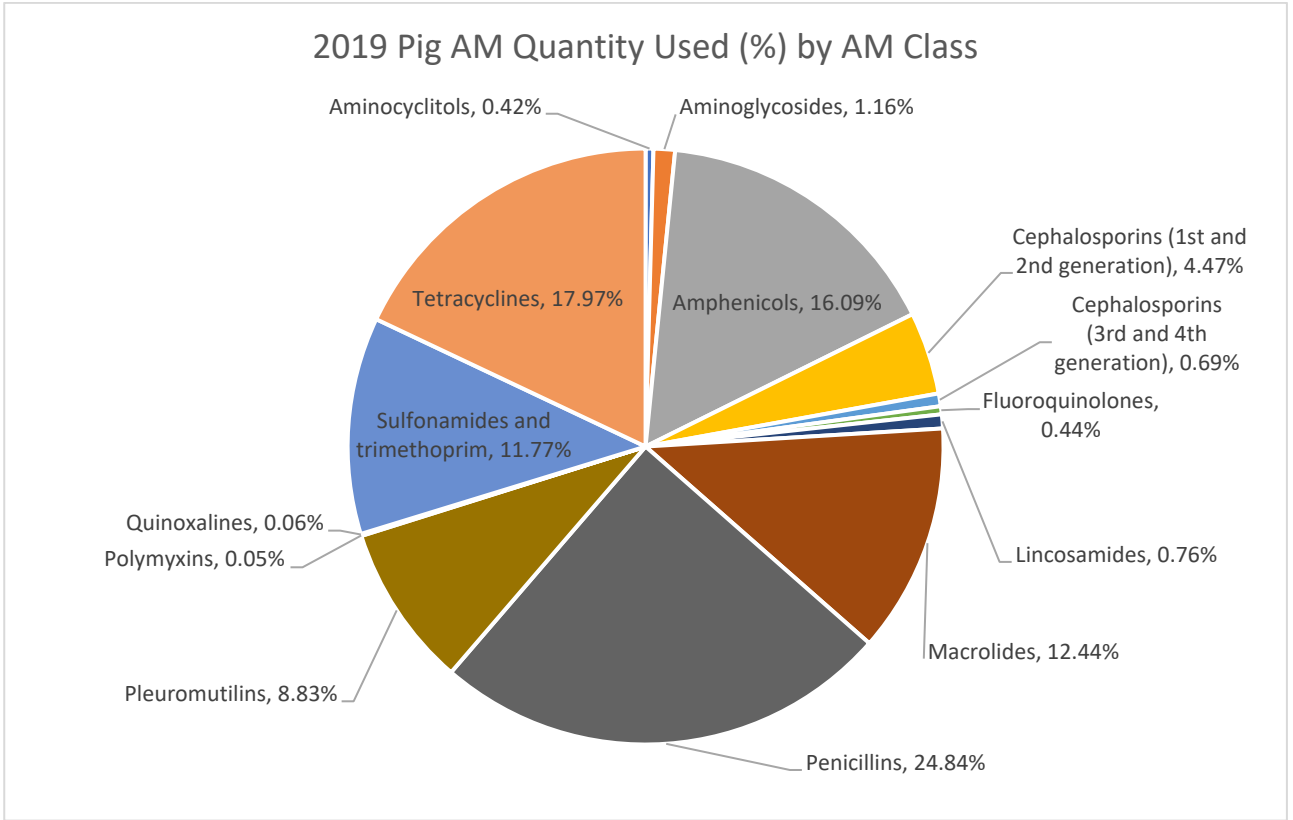


Table 2.4 provides details on the usage of major AM categories by number of farms, based on reports from 41 farms. Although there were 43 licensed farms in 2019, three of these are operated by the same owner and results have been combined as one farm. Three farms are inactive and two farms did not provide any reports in 2019 hence results are presented for a total of 36 farms. Given this is a voluntary reporting scheme the rate of reporting is regarded as excellent. Feed samples collected in 2018 from the two farms that did not report in 2019 did not contain any AMs. However, faecal waste collected from these two farms in 2019 contained low levels of tetracyclines (both farms) and traces of sulfonamides (one farm). These audit results suggest that these farms are not high level users of AMs and therefore the absence of reporting would not adversely affect information provided by other farmers on usage.

Table 2.4 Breakdown of AMU by number of pig farms reporting usage, range of quantities used and AMs used

Antimicrobial class	Farms reporting usage	Range of usage (mg/kg TAB) excluding zero usage	Main AMs used
Aminocyclitols	9/36	0.1-4	Spectinomycin
Aminoglycosides	15/36	0.01-11	Gentamycin, Kanamycin, Neomycin, Streptomycin
Amphenicols	23/36	0.18-133.5	Florfenicol
Cephalosporins (1st and 2nd Gen)	1/36	151	Cephalexin
Cephalosporins (3rd and 4th gen)	20/36	0.02-12.39	Cefquinome, Ceftiofur
Fluoroquinolones	12/36	0.17-3.97	Enrofloxacin, Ciprofloxacin
Linocosamides	17/36	0.1 – 10.3	Lincomycin
Macrolides	19/36	2.4-87.3	Tilmicosin, Tylosin, Tylvalosin
Penicillins	34/36	0.16-266	Amoxicillin, Ampicillin, Benzylpenicillin
Pleuromutilins	9/36	2.6-126.0	Tiamulin
Polymyxins	15/36	0.04-0.53	Colistin
Quinoxalines	5/36	0.02-5.19	Mequindox
Sulfonamides and Trimethoprim	10/36	0.14-97.61	Sulfadiazine, Sulfadimidine, Sulfamethoxazole, Sulfamonomethoxine and

			various combinations, including combination with Trimethoprim
Tetracyclines	23/36	0.12-187.4	Chlortetracycline, Doxycycline, Oxytetracycline

There was no known purposive use of AMs for growth promotion by pig farmers.

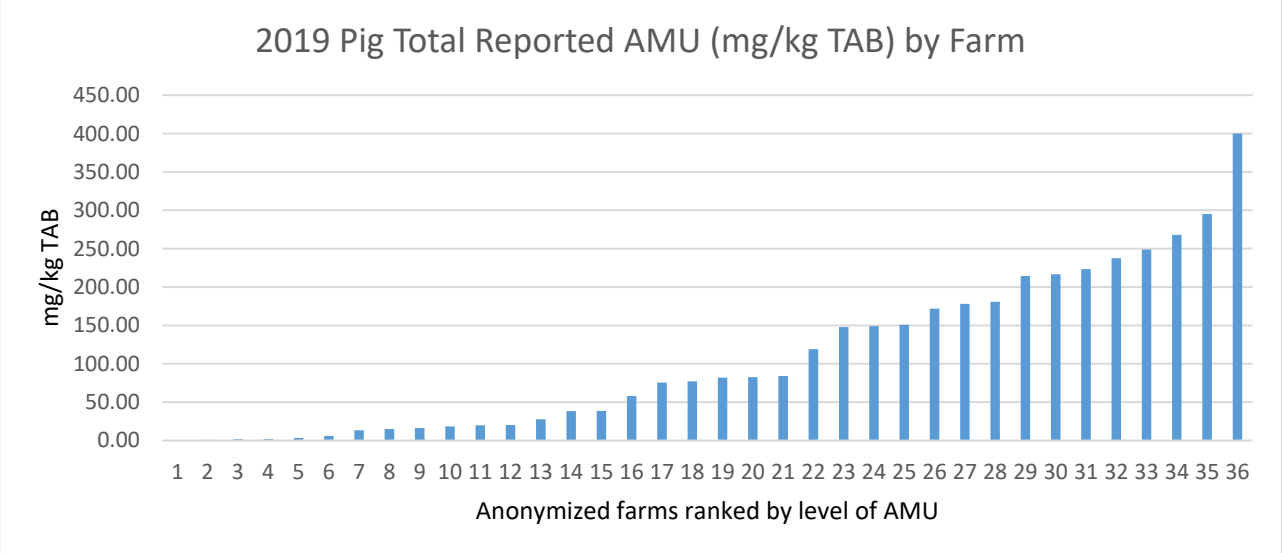
Of the WHO listed highest priority critically important AMs, 3rd and 4th generation cephalosporins, fluoroquinolones, polymyxins (colistin) were used in small quantities for therapeutic purposes. No quinolones were used in feed.

Macrolides are regarded by WHO as highest priority critically important AMs largely because of the effect they can have in driving resistance in *Campylobacter jejuni*, an organism that has not been cultured in chickens and pigs on Hong Kong farms in 2019 during this round of testing. Macrolides remain important AMs for treatment of respiratory and enteric disease in grower pigs.

Figure 2.8 shows the total reported AMU by pig farm and demonstrates considerable variation in the quantities used by individual farmers.

Total quantities used by mg/kg TAB provide benchmarking information for individual farms to see how they compare with other (anonymised) farms. AMU reports are prepared for each individual farm and the significance of results are discussed. The median usage was 80 mg/kg TAB whereas the mean was skewed upwards at 111 mg/kg TAB demonstrating that a few farms were relatively higher users than others.

Figure 2.8 Total reported AMU by pig farm (mg/kg TAB)



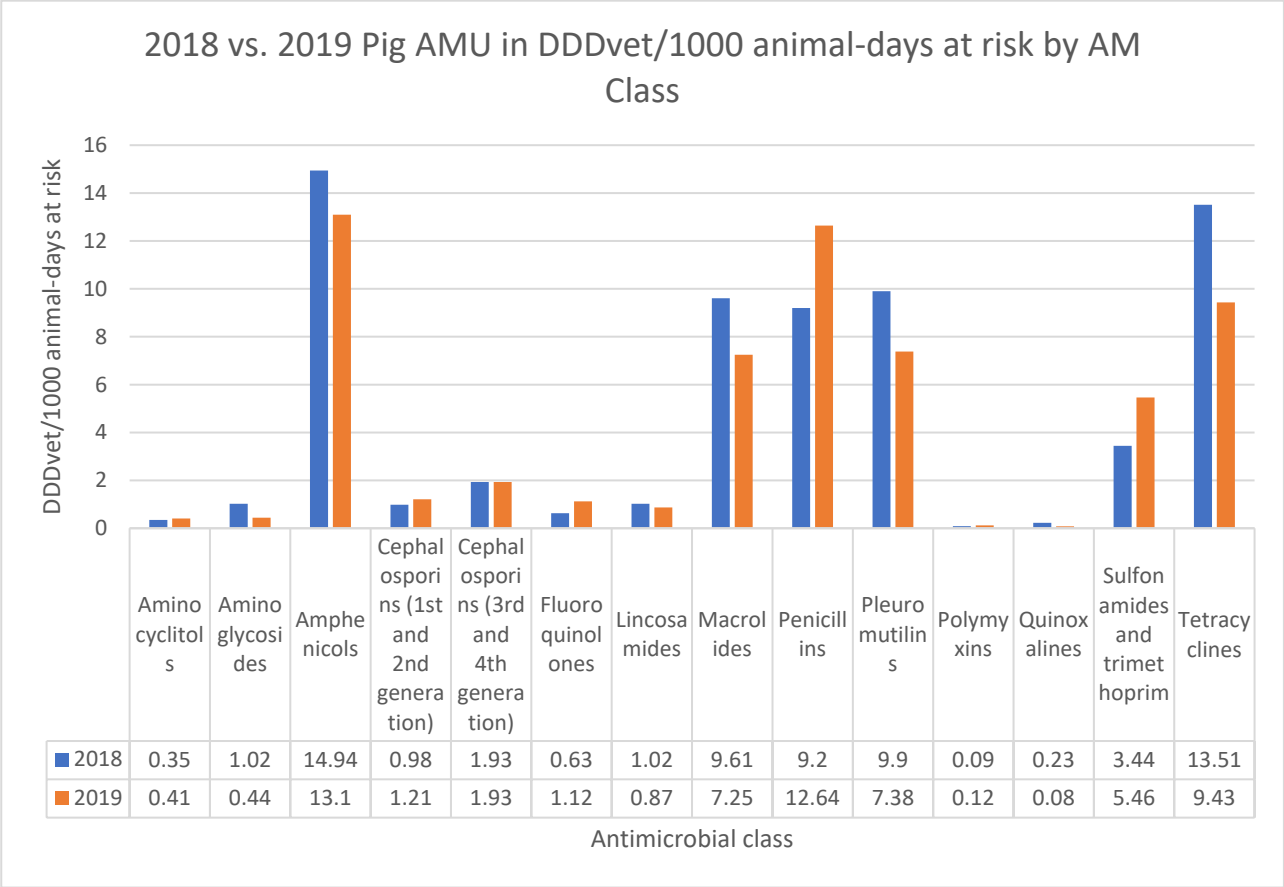
Results for AMU were collected on a trial basis in the last 7 months of 2018. A comparison between results for 2018 and 2019 demonstrates that there may have been an increase in usage of penicillins and a reduction in usage of tetracyclines and amphenicols.

Fig 2.9 and Fig 2.10 demonstrate total reported usage by AM class in mg/kg target animal biomass (TAB) and in DDDvet/1000 animal-days at risk.

Figure 2.9 AMU by mg/kg TAB in pig production in 2018 and 2019

	Aminocyclitols	Aminoglycosides	Amphenicols	Cephalosporins (1st and 2nd generation)	Cephalosporins (3rd and 4th generation)	Fluoroquinolones	Lincosamides	Macrolides	Penicillins	Pleuromutilins	Polymyxins	Quinoxalines	Sulfonamides and trimethoprim	Tetracyclines
2018	0.31	3.08	19.77	3.89	0.74	0.27	0.95	18.51	19.42	12.76	0.05	0.22	8.96	25.94
2019	0.47	1.29	17.89	4.97	0.77	0.49	0.84	13.84	27.62	9.82	0.05	0.07	13.08	19.98

Figure 2.10 AMU by DDDvet/1000 animal-days at risk in pig production in 2018 and 2019



	Aminocyclitols	Aminoglycosides	Amphenicols	Cephalosporins (1st and 2nd generation)	Cephalosporins (3rd and 4th generation)	Fluoroquinolones	Lincosamides	Macrolides	Penicillins	Pleuromutilins	Polymyxins	Quinoxalines	Sulfonamides and trimethoprim	Tetracyclines
2018	0.35	1.02	14.94	0.98	1.93	0.63	1.02	9.61	9.2	9.9	0.09	0.23	3.44	13.51
2019	0.41	0.44	13.1	1.21	1.93	1.12	0.87	7.25	12.64	7.38	0.12	0.08	5.46	9.43

2.1.3 Reported usage of antimicrobials on fish farms

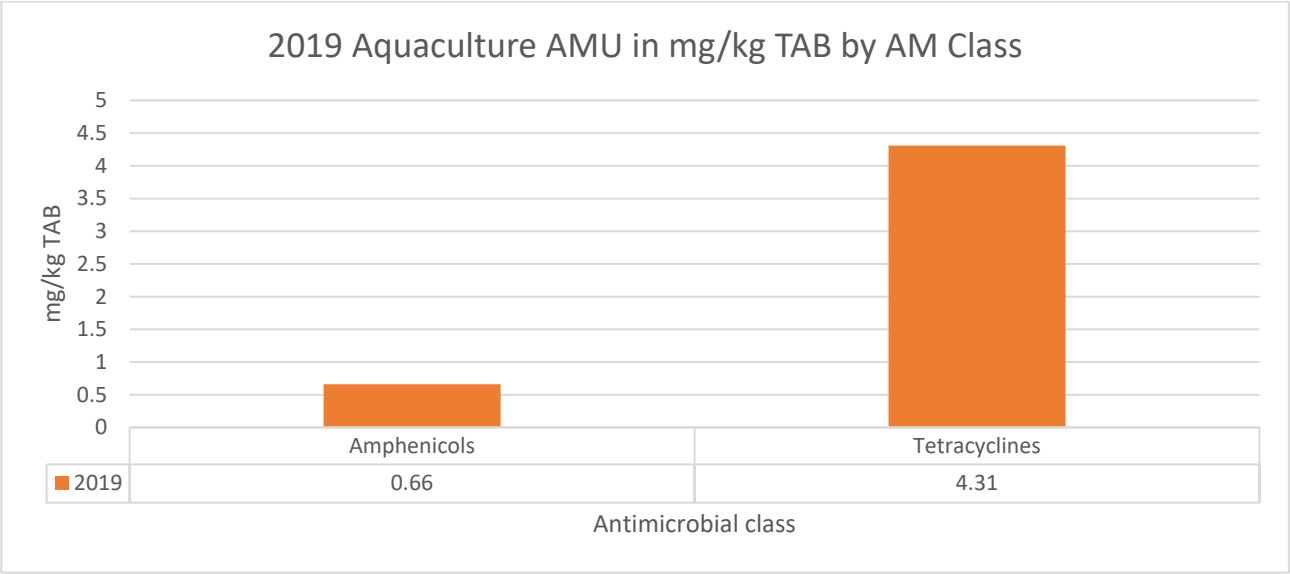
Fish farmers reported limited usage of AMs in 2019. This is consistent with findings from field visits and disease investigations that suggest most bacterial diseases are secondary to other factors. Control of the environment and other pathogens (especially external parasites) minimises the need for use of AMs.

A mandatory AM usage reporting system for registered fish farms under AFCD's Accreditation Fish Farm Scheme (AFFS) has been in place since 2005, and a voluntary AM usage reporting system for other fish farms has been in place since May 2017.

Usage of WHO highest priority critically important AMs is not reported in fish farms.

Total reported usage in 2019 was 15.73 kg with only tetracyclines and florfenicol used.

Figure 2.11 AMU by mg/kg TAB in aquaculture in 2019



A total of 4.97 mg AM/kg TAB of fish produced in local pond fish and marine fish farms was reported.

2.2 Audit testing for AMU

The AMU reported above relies on voluntary reporting by farmers supplemented by collective information from veterinarians servicing these industries. To ensure that the reports from farmers reflect actual usage and intake of antimicrobials an auditing system is being established for chicken and pig farms, based on tests of faecal waste from farms. A system for fish farms has been in place for a number of years, especially for accredited farms.

During 2019 the system of auditing using faecal waste was being developed. As a trial, a small number of samples were assessed (eight samples – four from pig and four from chicken farms). Of these, low quantities of AMs, generally in line with those used on the farm were detected. This programme has been expanded in 2020.

Additional testing of feed supplemented the auditing system and was used in 2018 and 2019. This testing was introduced to provide information to farmers on inadvertent usage of AMs as a result of undeclared incorporation of AMs into feed or carryover of AMs between batches of feed, a recognised issue (FAO 2019). During the consultation process when developing the AM usage scheme, the pig and chicken industries indicated that they were not knowingly using antimicrobials as growth promoters. However, before announcing this stance publicly they wanted to know

whether there was any inadvertent inclusion of antimicrobials in feed that was purchased/used. As a result, testing of selected feeds was undertaken (see Annex 2 for methods used for sample selection).

It is recognised across much of Asia that labels for purchased feed do not always provide information on the AMs that they contain. In addition, some antimicrobials can carry over between batches of feed, both in commercially prepared rations and home-mixed feed. Most farms in Hong Kong that produce their own feed only have one mixer for all feeds and due to the electrostatic nature of some antimicrobials, carryover between feed batches can occur.

Use of feed testing results

Chicken farms

When feed testing began in 2018, two chicken farms were found to have feed that contained moderate levels of zinc bacitracin. Farmers were advised of the finding. When feed samples were collected again from these farms in 2019 no zinc bacitracin was detected.

Only two chicken farms did not provide data on AMU during 2019 but one of these provided feed samples that were found to contain oxytetracycline (c.100mg/kg), providing evidence of AM usage on the farm. In follow up discussions, the farmer indicated that around the time of sample collection, he used in-feed tetracyclines for a disease outbreak.

Feed testing revealed that, of the 24 chicken farms from which samples were collected, six had no detectable antimicrobials in the feed samples tested.

Seven of 24 farms had very low levels of a range of AMs (most likely due to carryover), especially but not only kitasamycin and related chemicals (see below), oxytetracycline, sulfonamides and penicillins. Of these samples, one had low levels of colistin (<2mg/kg) two had low levels of zinc bacitracin (c. 5 mg/kg and <5mg/kg – below the level of quantitation). Investigations into the reason for the presence of these AMs are continuing but in all cases the farmers had no knowledge of purposely using these drugs.

Based on results of feed testing it was apparent that nine farms were, at least at the time the samples were collected, providing feed that contained AMs at levels typically used for therapy of disease. Of these eight had not previously reported usage.

Samples from five farms were found to contain virginiamycin (2.4 to 44 mg/kg and one that could not be quantified). For feed containing kitasamycin, two clusters were detected. One cluster, comprising four farms had low levels in feed. The farms were linked to one farm that produced and supplied feed for the other farms. The other cluster of five farms had higher levels and the farms used feed from the same company. In both cases farmers were surprised to find that their feed contained these AMs.

Pig farms

In 2019, of the 44 feed samples tested, 21 did not contain any detectable antimicrobials, 15 contained trace to very low levels of AMs suggestive of carryover. One sample contained amoxicillin at 18 mg/kg.

Seven had relatively high levels of AM – predominantly chlortetracycline (c.100 mg/kg), amoxicillin and one florfenicol. The farms from which these samples were collected reported usage of these AMs in their voluntary reports.

Feed testing will be phased out gradually as faecal waste testing is fully implemented.

Legislation on antimicrobials in animal feed

Currently there is limited legislative control on use of antimicrobials in feed for livestock. The Antibiotics Ordinance (Cap 137) does not apply to antibiotics in animal fodder.

The Public Health (Animals and Birds) Chemical Residues Regulation (Cap 139N), introduced in 2001, prohibits the use of avoparcin and chloramphenicol in food animals. It prevents the supply of any fodder which contains or is mixed with any agricultural and veterinary chemical unless it lists all the agricultural and veterinary chemicals contained or mixed in the fodder and their respective amounts; the instructions for use of the fodder; and the withholding period (regulation 14).

Urine samples are collected from pigs at slaughter to test for antimicrobials as part of residue monitoring and for preventing pigs administered illegal chemicals from entering the food chain. Samples of urine are collected from all batches of pigs on arrival in the slaughterhouse. This provides an addition audit check on AM usage. In 2019, a total of 9873 urine specimens collected from pigs as they entered the slaughterhouse were tested (using ELISA screening tests) for presence of

chloramphenicol. Of these, 601 samples were tested for tetracyclines, sulphonamides, beta-lactams, streptomycin, gentamicin, neomycin, tylosin, macrolides, fluoroquinolones, flumequine, virginiamycin, furazolidone and furaltadone (see Annex 2 Methods). Three samples gave a positive result for sulphonamides. One of these three farms of origin was known to use sulphonamides and the other two farms have been investigated. The possibility of carryover of sulphonamides in commercial feed cannot be ruled out.[2]

Collection and testing of audit samples to detect unreported or inadvertent AM usage in fish farms have commenced. This includes fish, fish feed, water, and sediment samples. No AMs were detected.

Usage of other chemicals in pig and chicken feed

Feed testing in 2019 demonstrated high levels of zinc (exceeding EU recommended levels) in 31 pig feed samples and 14 chicken feed samples and high levels of copper in 20 pig feed and 17 chicken feed.

2.3 Discussion on AM Usage

The system adopted for assessing usage of AMs on farms in Hong Kong differs from that in most other places due to the absence of sales data. It is expected that methods for collection of data will evolve over time as farms move to supply of AMs by prescription only. These data on AM usage, although imperfect, can be used as a base line for measuring changes in practices over time given action to reduce AM usage on farms is relatively recent. However, it will be necessary to take into account changes that are made in the manner in which data are collected.

The quantities of AM reported as being used on most chicken farms in Hong Kong SAR are low. It was found that two farms were responsible for much of the reported AM usage. By targeting disease control and stewardship programmes at these farms there are good prospects for reducing further the usage of AMs in chicken production.

As a rule, chicken producers use less AM than pig producers due, in part, to the shorter life span of their birds and the manner in which they are reared.

[2] See FAO and WHO. 2019. Carryover in feed and transfer from feed to food of unavoidable and unintended residues of approved veterinary drugs. Report of the Joint FAO/WHO expert meeting – 8–10 January 2019, FAO Headquarters, Rome, Italy. FAO Animal Production and Health Report No. 13. Rome, Italy

In pigs, quantities used are higher than those in other countries with long standing AM usage programmes (e.g. UK, Netherlands and Denmark). Overall, they are likely to be below those in some major pig producing nations such as Spain and Italy (e.g. see ESVAC report for 2018). Note that results from different countries are not directly comparable given different systems for data recording and for production. Nevertheless, the major differences, compared to other countries, are a greater use of amphenicols (florfenicol) and macrolides. Levels of use of fluoroquinolones in pigs appear to be below those used in some European countries involved in pig production.

Based on information collected from pig farmers, AMs were mainly used for treatment of enteric and respiratory diseases with some usage in periparturient sows to control and treat post-partum dysgalactia and other related reproductive conditions.

As with chicken farms, considerable variation in usage was seen between pig farms. Targeting of measures to farms with higher usage should result in improvements across the industry. Because of the small number of farms one or two outliers can influence the collective results as seen by the difference between the mean and median total usage figures.

Fish farms are not significant users of AMs and there appears to be little scope to reduce levels used in this sector.

All pig and chicken farms have been given copies of reports demonstrating their usage compared with other farmers in Hong Kong SAR covering total usage and usage by antimicrobial agent. This will also be used as a tool for helping farmers understand better their relative rates of usage and to assess ways to reduce usage.

2019 was a year for consolidating information on AM usage in farms in Hong Kong SAR. As the farm veterinary services provided by City University continue to develop and there is a shift over time to supply of AMs by veterinary prescription only (eventually replacing the current antibiotic permit system) it is expected that fluoroquinolones, 3rd and 4th generation cephalosporins and colistin will only be prescribed on the basis of susceptibility testing that demonstrates they are the only alternative available for treatment of sick animals. More information is required on susceptibility patterns of pathogens to determine whether these AMs are required. Data in this area will continue to be collected.

Despite conducting feed audits it is possible that some usage of in-feed medication was not reported, in part because farmers did not know the feed was medicated and also because some farmers only reported use of medications that they added to feed. Note also that at present there are few legal instruments for controlling usage of antimicrobials in feed as described above in the section on audit testing. Changes should be considered to existing regulations on antibiotics in feed.

3. Antimicrobial Susceptibility Testing

3.1 Background

As with the AMU monitoring programme, the small size of the Hong Kong pig and poultry production sectors means that systems designed for Hong Kong differ from those in places with many farms. Samples for this program are collected on farms rather than in the slaughterhouse or markets to reflect events that are occurring just prior to sale – differences have been noted in AMR patterns in samples between pigs on arrival and after being in a slaughterhouse lairage (Feng et al. 2021). Market and slaughterhouse sampling covering imported and local food animals, which has been conducted by Hong Kong University on behalf of Department of Health for over 10 years, is also continuing. This work and other work conducted previously have highlighted the high levels of resistance in commensal organisms and opportunistic pathogens and has also identified the genetic basis of resistance for a number of organisms from farm animals reared in or imported to Hong Kong SAR (see, for example, Cheng et al. 2015, Ho et al. 2010, Ho et al. 2015, Ho et al. 2018). Similar findings have been reported in the broader region (e.g. Nuangmek et al. 2018, Zhang et al. 2017).

AMR monitoring in national/regional programmes covering pigs and chickens (such as the National Antimicrobial Resistance Monitoring System [NARMS] in the USA and those in the EU [e.g. the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme, DANMAP]), is based largely on phenotypic testing of a small number of indicator organisms followed by genetic testing of selected isolates for resistance genes. Usually, the organisms chosen are well characterised commensals, especially *Escherichia coli* and, in some cases, *Enterococcus* spp. and potential zoonotic agents, in particular *Salmonella* and *Campylobacter* spp. Animal pathogens are also included, but the number of organisms available for assessment can limit their value if relying only on routine diagnostic specimens. The same strategy has been applied for monitoring in Hong Kong SAR.

The system was trialled in 2018 and officially commenced in mid-2019. The results provide a wealth of information on AM susceptibility in pig, chicken, and fish farms in Hong Kong SAR and, in many cases, the mechanisms of resistance. These results provide a basis on which changes over time in resistance patterns and genes associated with reduced susceptibility to antimicrobials can be assessed.

Main findings from AM susceptibility testing

Escherichia coli (indicator organisms) – pigs and chickens

- High percentage of strains (>60%) "resistant" to tetracyclines, aminopenicillins, sulfonamides, streptomycin in line with observations in other studies in the region
- Considerable resistance to fluoroquinolones in chickens despite apparent absence of use (apparently higher than in pigs where usage on some farms continues)
- No apparent link between usage of fluoroquinolones and resistance in pigs (comparing farms using vs. not using)
- No resistance detected to some important reserve classes of AM such as carbapenems and combinations such as piperacillin/tazobactam
- Very low resistance to colistin (one pig and two chicken isolates); the chicken isolates belong to *E. coli* ST648, a globally emerging multidrug resistant strain
- Trend of resistance to a larger number of AM classes for *E. coli* grown on selective media
- Approximately 25% of *E. coli* from non-selective media resistant to <3 AM classes
- Persistence of *E. coli* resistant to 3rd generation cephalosporins in chickens despite only one farm known to be using these AMs

Salmonella in pigs and chickens

- 21 isolates from pig farms
- 24 isolates from chicken farm environmental swabs
- Overall lower level of resistance compared with *E. coli,* especially chicken *E. coli*
- Several multidrug resistant (MDR) strains of global significance, suggesting resistance pattern did not develop locally

Campylobacter spp. in pigs and chickens

- Low number of *Campylobacter coli* isolates (8), all from pigs; no *Campylobacter jejuni* isolated
- 100% resistance to fluoroquinolones consistent with findings elsewhere in Asia
- Several multidrug resistant isolates
- The gene *optR* was detected in five isolates

Enterococcus spp. in pigs and chickens

- No vancomycin-resistant organisms

Fish

- Baseline data collected on *Vibrio* spp., *Photobacterium damselae* and *Aeromonas* spp. from skin mucus
- No evidence of unusual patterns of resistance, consistent with low levels of AM usage

3.2 Results

In 2019, 68 samples from 34 pig farms were cultured and results analysed.

Fifty-two sets of samples from 26 chicken farms were cultured and analysed.

Specimens from pigs were faecal samples. Samples from chickens were cloacal swabs and drag swabs for *Salmonella* (see Methods in Annex 2).

Note that for commensal *Escherichia coli* and *Salmonella* from chickens a new, comprehensive customised MIC plate was adopted for use for approximately half of the isolates. It was not used for isolates from pigs in 2019. This new plate has also been used for all isolates from 2020.

3.2.1 Commensal *Escherichia coli*

Pigs

From the pig samples, 126 commensal *Escherichia coli* were selected for susceptibility testing. Of these 65 (from 34/34 farms) were isolated on non-selective media and 61 (from 32/34 farms) on media designed to select for strains resistant to extended spectrum cephalosporins. No *E. coli* grew on selective media for carbapenem-resistant organisms. Whole genome sequence results from 11 selected pig isolates were analysed.

Results of susceptibility tests are summarised in Table 3.1. Full minimum inhibitory concentration (MIC) profiles, including values used for cut off points are provided in Annex 1 Tables A1.1 to A1.3.

Table 3.1 Percentage of commensal *Escherichia coli* from pigs exceeding the MIC breakpoint ("R")[@]

Antimicrobials	Non-selective media (n=65)	Selective media (n=61)	All (n=126)
Amoxicillin/Clavulanic acid	6.2%	18%	11.9%
Ampicillin	64.6%	100%	81.7%
Azithromycin[@]	3.1%	14.8%	8.7%
Cefoxitin	3.1%	14.8%	8.7%
Ceftazidime/Avibactam	0%	0%	0%
Ceftiofur	1.5%	90.2%	44.4%
Ceftolozane/Tazobactam	0%	0%	0%
Ceftriaxone	7.7%	100%	52.4%
Chloramphenicol	63.1%	73.8%	68.3%
Ciprofloxacin	9.2%	23.0%	15.9%
Colistin	1.5%	0%	0.8%
Gentamicin	10.8%	21.3%	15.9%
Meropenem	0%	0%	0%
Nalidixic acid[@]	26.2%	41.0%	33.3%
Piperacillin/Tazobactam	0%	0%	0%
Streptomycin[@]	55.4%	70.5%	62.7%
Sulfisoxazole	58.5%	78.7%	68.3%
Tetracycline	76.9%	86.9%	81.7%
Trimethoprim/Sulfamethoxazole	43.1%	54.1%	48.4%

[@]No CLSI (Clinical & Laboratory Standards Institute) or EUCAST (European Committee on Antimicrobial Susceptibility Testing) clinical break point. NARMS value used.

Chickens

From the chicken samples, 94 commensal *Escherichia coli* were selected for susceptibility testing. Of these, 48 (from 26/26 farms) were isolated on non-selective media and 39 (from 19/26 farms) on media designed to select for strains resistant to 3rd and 4th generation cephalosporins. Seven *E. coli* (from 5/26 farms) grew on selective media for carbapenem-resistant organisms. Whole genome sequencing results from 17 selected chicken isolates were analysed. Results of susceptibility testing are summarised in Table 3.2 and full MIC profiles are provided in Annex 1 Tables A1.4 to A1.6.

Table 3.2 Percentage of commensal *Escherichia coli* from chickens exceeding the MIC breakpoint ("R")[@]

Antimicrobial	Non-selective media (n=48*)	Selective media (n=46*)	All (n=94*)
Amikacin**	0%	0%	0%
Amoxicillin/Clavulanic acid	0%	2.2%	1.1%
Ampicillin	70.8%	100%	85.1%
Azithromycin[@]	0%	2.2%	1.1%
Cefepime**	3.1%	18.8%	10.9%
Cefotaxime**	25%	90.6%	57.8%
Cefoxitin	0%	2.2%	1.1%
Ceftazidime**	0%	18.8%	9.4%
Ceftazidime/Avibactam*	0%	0%	0%
Ceftiofur	22.9%	93.5%	57.4%
Ceftolozane/Tazobactam*	0%	0%	0%
Ceftriaxone	18.8%	95.7%	56.4%
Chloramphenicol	50.0%	73.9%	61.7%
Ciprofloxacin	27.1%	50.0%	38.3%
Colistin	0%	4.3%	2.1%
Florfenicol[@]**	34.4%	62.5%	48.4%
Fosfomycin**	0%	18.8% (n=7/32)	9.4%
Gentamicin	29.2%	34.8%	31.9%
Imipenem**	0%	0%	0%
Levofloxacin**	40.6%	59.4%	50.0%
Meropenem	0%	0%	0%
Nalidixic acid[@]	37.5%	76.1%	56.4%
Nitrofurantoin**	0%	0%	0%
Piperacillin/Tazobactam	0%	0%	0%
Streptomycin[@]	64.6%	89.1%	76.6%
Sulfisoxazole	68.8%	73.9%	71.3%
Temocillin**	0%	0%	0%
Tetracycline	77.1%	84.8%	80.9%
Tigecycline**	3.1% (n=1/32)	0%	1.6%
Trimethoprim/Sulfamethoxazole	56.3%	54.3%	55.3%

*Due to introduction of a customised plate with an increased range of AMs during 2019 not all samples were tested against all AMs in this table. Those with a single asterisk were discontinued once the new customised plate was introduced.

**Those with two asterisks were added via the customised MIC plate.

[@]No CLSI or EUCAST clinical breakpoint. NARMS value used if available or EUCAST non-wild type.

The following section discusses susceptibility patterns and related genes against important AM classes.

3.2.1.1 Aminoglycosides

A higher proportion of chicken isolates than pig isolates exceeded the CLSI breakpoint for gentamicin.

Sixteen genes associated with aminoglycoside resistance were detected in strains of *E. coli* that were sequenced including *aac(3)-IId, aac(3)-IIa, aac(3)-IV, aac(6')-Ib-cr, aadA1, aadA2, aadA2b, aadA22, aadA5, aadA, ant(2'')-Ia, aph(3')-Ia, aph(3')-IIa, aph(3'')-Ib (strA), aph(4)-Ia, aph(6)-Id (strB)*. Presence of *aph(3'')-Ib* and, *aph(6)-Id* in isolates that were sequenced corresponded well with streptomycin MICs that exceeded the NARMS cut off point. Organisms resistant to gentamicin usually possessed genes encoding for N-Acetyltransferases such as *aac(3)II-d*. In some organisms, multiple aminoglycoside resistance genes were detected (see Poerel et al. 2018 and Ho et al. 2010).

3.2.1.2 Beta-lactams

Penicillins

64.6% of pig isolates and 70.8% of chicken isolates exceeded the CLSI breakpoint for ampicillin.

As expected, 100% of isolates grown on extended-spectrum beta-lactamase (ESBL)-selective media exceeded the CLSI breakpoint for ampicillin.

The main genes found (other than $bla_{\text{CTX-M}}$) were within the bla_{TEM}-type especially $bla_{\text{TEM-1B}}$.

Beta-lactams with beta-lactamase inhibitors

A lower proportion of chicken isolates than pig isolates exceeded the CLSI breakpoint for amoxicillin/clavulanic acid. The AmpC gene $bla_{\text{CMY-2}}$ was detected in 3/6 phenotypically resistant organisms that were sequenced.

No isolates exceeded breakpoints for piperacillin/tazobactam (chickens only, this combination was not tested for pig isolates).

3rd and 4th generation cephalosporins and cephamycins

For *E. coli* isolates that were recovered on non-selective media, more chicken isolates than pig isolates were resistant to ceftiofur (see Table 3.3) despite the fact that only one chicken farm is known to be using cephalosporins. This finding was unexpected and demonstrates that linkage between current usage of AMs and AMR patterns is not clear-cut. Once resistance genes are present they can persist, even in the absence of direct selection pressure.

Table 3.3 Percentage of resistance for 3rd and 4th generation cephalosporins and cephamycins for *E. coli* isolates from pigs and chickens (non-selective media)

Antimicrobial	Chicken (n=48)	Pig (n=65)
Cefoxitin	0%	3.1%
Ceftiofur	22.9%	1.5%
Ceftriaxone	18.8%	7.7%
Ceftazidime	0%*	ND
Cefotaxime	25.0%*	ND
Cefepime	3.1%*	ND

*n=32

For *E. coli* recovered on selective media for ESBL-producing bacteria, resistance rates >90% against ceftiofur were detected. Lower levels of resistance were detected against cefepime and ceftazidime in chickens (isolates from pigs in 2019 were not tested against these AMs).

Likely ESBL-producing *E. coli* (resistant to ceftiofur) were isolated on non-selective media from 31% (8/26) of chicken farms and 3% (1/34) of pig farms. On selective media they were isolated from 81% (21/26) of chicken farms and 88% (30/34) of pig farms.

Resistance to potentiated cephalosporins was not detected.

All isolates sequenced that exceeded the cut off point for ceftiofur possessed ESBL/AmpC genes. The main ESBL/AmpC genes detected in isolates from 2019 that were sequenced were $bla_{\text{CMY-2}}$ (3 pig isolates) $bla_{\text{CTX-M-14}}$ (1 pig and 2 chicken isolates) $bla_{\text{CTX-M-15}}$ (1 pig isolate and 2 chicken isolates – both were ST648 – see text box), $bla_{\text{CTX-M-55}}$ (7 chicken isolates) and $bla_{\text{CTX-M-65}}$ (2 pig and 2 chicken isolates).

One pig isolate that was resistant to cefoxitin was found to carry the $bla_{\text{DHA-1}}$ gene.

3.2.1.3 Carbapenems

No phenotypic resistance was detected to meropenem or imipenem in the strains of *E. coli* isolated, including those on media selective for carbapenem-resistant isolates. Genes that have been associated with carbapenem resistance in some bacterial species, in particular bla_{OXA-10}, were found in multiple isolates from pigs (3) and chickens (5). Phenotypically these organisms were all susceptible to meropenem, as has been described elsewhere (Antunes et al. 2014).

Carbapenem-resistant *E. coli* have been detected by others in Hong Kong SAR in pigs in a slaughterhouse (e.g. Ho et al. 2018).

3.2.1.4 Phenicols (chloramphenicol, florfenicol)

Resistance to phenicols was >50% in *E. coli* isolates from both chickens and pigs, with a lower proportion of chicken isolates with reduced susceptibility against florfenicol than chloramphenicol (pig isolates were not tested against florfenicol in 2019). In isolates that were sequenced, reduced susceptibility was associated mainly with the presence of other relevant resistance genes that were also detected in some isolates, including *catA2* and *cmlA1*. Florfenicol is used commonly on pig farms but not chicken farms. Higher rates of resistance to chloramphenicol were apparent in isolates from pigs than those from chickens. Organisms grown on ESBL-selective media appeared to have higher rates of resistance to chloramphenicol than those grown on non-selective media.

3.2.1.5 Fluoroquinolones

Resistance to quinolones appeared to be higher in chicken isolates than those from pigs, despite the fact that chicken farmers are not known to be using or reporting usage of fluoroquinolones. The proportion resistant to quinolones was higher in organisms isolated on ESBL-selective media than non-selective media. When *E. coli* from pig farms using fluoroquinolones were compared with those from farms not using fluoroquinolones there did not appear to be any difference in the proportion of resistant organisms.

Quinolone resistance genes detected in isolates that were sequenced included mutations in *gyrA* (S83L, D87N), *parC* and *parE*. Other relevant genes detected included *qnrS1, qnrS2, qnrB4, oqxA and oqxB*.

3.2.1.6 Tetracycline

Resistance to tetracyclines was high with a possible increase in organisms grown on ESBL-selective media compared to non-selective media. Pig and chicken isolates had similar rates of resistance. The main genes detected for tetracycline resistance belong to the *tet* family (especially *tetA,* but also *tetB* and *tetM*).

3.2.1.7 Polymyxins

One isolate from pigs was found to be resistant to colistin. It possessed a plasmid-borne *mcr1.1* gene. This strain, belonging to ST453, was also resistant to gentamicin (*aac(3)-IId*), ampicillin ($bla_{\text{TEM-1a}}$) chloramphenicol (*floR*), and ciprofloxacin (dual mutations in *gyrA*).

Two isolates from chickens resistant to colistin were detected on two farms. These belong to ST648, an emerging multi-drug resistant (MDR) lineage (see text box).

Multidrug-resistant *Escherichia coli* ST648

Background

Some antimicrobial resistant strains of *Escherichia coli* that can cause disease in humans and animals as opportunistic pathogens have become or are becoming "pandemic".

The best known are certain strains within *E. coli* multilocus sequence type 131 (ST131) (Whitmer et al. 2019). They are an important cause of disease in humans, both as uropathogens and invasive organisms. Another sequence type, ST648, is increasingly being recognised as an important emerging "pandemic" strain (Johnson et al. 2017, Schaefler et al. 2019). Both are known to carry genes encoding for extended spectrum beta-lactamases, including $bla_{\text{CTX-M-15}}$. They are also resistant to a number of other antimicrobials.

"*ESBL-producing* E. coli *are leading MDR pathogens, headed by a few internationally relevant, high-risk clonal lineages with ST131 as the most prominent. ST648 presents an emerging lineage increasingly reported from multiple origins with the greatest potential to follow ST131's success*" (Schaefler et al. 2019).

Two (very similar) extensively drug resistant *E. coli* ST648 have been isolated in AMR surveillance from two chicken farms by selective culture for cephalosporin-resistant organisms. Neither farm is known to use antimicrobials on a regular basis and reported no AM usage in 2019. A feed sample from one farm contained

kitasamycin (22mg/kg) and one from the second farm contained c.6mg/kg of zinc bacitracin. Neither were known feed inclusions by the farmer.

These organisms carried at least 19 different resistance genes, including genes encoding for resistance to rifampicin and had chromosomal changes indicative of fluoroquinolone resistance. Phenotypically they were resistant to aminoglycosides, amphenicols, beta-lactams, 3rd and 4th gen cephalosporins, colistin, tetracycline and quinolones. Relevant genes were present to explain the resistance to each of these, including *bla*$_{\text{CTX-M-15}}$ and *mcr1.1*. These organisms have almost certainly been introduced to the farm and various pathways exist for their entry, including contaminated feed and farm workers.

ST648 is present already in humans (see, for example, Paulshus et al. 2019) and wild birds (Mukerji et al. 2020) so their detection in chickens is just one part of a much larger issue. Nevertheless, if these organisms are found in chickens (in which they are able to colonise the gut) there is potential for contamination of meat during processing. Note that these organisms can persist even without pressures of antimicrobial usage. There is no fitness cost for the resistance pattern and some believe links to other genes such as those that promote formation of biofilms may give them a competitive advantage. It is considered likely that these were imported to the farm as multidrug resistant organisms.

The two chicken isolates detected were phenotypically sensitive to nitrofurantoin and fosfomycin that are used for treatment of AM-resistant urinary tract infections in humans. No resistance genes for these two AMs were detected in these isolates.

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3.2.1.8 Fosfomycin

A small number of fosfomycin resistant organisms were detected in chickens, all in organisms grown on ESBL-selective media. Pig isolates were not tested against fosfomycin in 2019. Two genes associated with fosfomycin resistance were detected – the recently recognised *fosA7* (Rheman et al. 2017) and *fosA3* (Chan et al. 2014) that has been described previously in Hong Kong in both animals and humans. One chicken *E. coli* possessed *fosA7* but was phenotypically sensitive. Further studies will be done on the genetics of these bacteria.

3.2.1.9 Macrolides

Limited evidence of resistance to macrolides was evident in *E. coli* isolates despite their widespread usage in pig farms (but see results for *Campylobacter* spp.). The gene *mph(A)* was detected in isolates that were sequenced that had high MICs for azithromycin – above the NARMS cut off point (see Gomez et al. 2019).

3.2.1.10 Sulphonamides and trimethoprim

High levels of resistance were detected with no apparent differences between species or organisms grown on ESBL-selective media or non-selective media. Lower levels of resistance were apparent for sulfamethoxazole/trimethoprim than for sulphonamide alone. The main genes associated with phenotypic resistance to trimethoprim detected were various *dfrA* genes (*dfrA1*, *dfrA7*, *dfrA12*, *dfrA14*, *dfrA17*) and, for sulfonamides, *sul1*, *sul2* and *sul3*.

3.2.2 Multidrug resistance in commensal *Escherichia coli*

The following tables provide information on the number and percentage of AM classes against which individual *E. coli* isolates were resistant (exceeding breakpoints) based on the criteria described by Magiorakis et al. 2012 (see Annex 2 Methods).

Approximately three quarters of *E. coli* isolated on non-selective media in both pigs and chickens are regarded as multidrug resistant (resistance to ≥3 AM classes). Four *E. coli* isolated from a pig on selective media were resistant to 8 classes of AM. The median number of classes for which resistance was present for pig and chicken isolates grown on non-selective media was 3 and 4, respectively.

As expected, isolates on ESBL-selective media were resistant to a greater number of classes than those on non-selective media (given these will always be resistant to ampicillin and usually 3rd generation cephalosporins). There is also likely some co-selection if multiple resistance genes are located on the same plasmid harbouring an ESBL gene.

Most of the resistance was against antimicrobials that have been used for many years, or used historically, including tetracyclines, sulfonamides, ampicillin, streptomycin and, for pigs, chloramphenicol (probably due to usage of florfenicol).

One of the isolates from pigs that exceeded clinical breakpoints for eight AM classes exceeded MIC breakpoints for ampicillin, amoxicillin/clavulanic acid, cefoxitin, ceftiofur, ceftriaxone, sulfisoxazole, trimethoprim/sulfamethoxazole, tetracycline, chloramphenicol and ciprofloxacin. It also exceeded NARMS cut off points for streptomycin, azithromycin and nalidixic acid. Whole genome sequencing revealed the resistance genes *aph(3'')-Ib*, *aph(6)-Id*, $bla_{\text{CTX-M-14}}$, $bla_{\text{DHA-1}}$, *mph(A)*, *floR*, *sul1*, *sul2*, *tetA*, *dfrA7*, *qnrB4* as well as single mutations in chromosomal genes *gyrA* and *parE*. The genes present provide a good match/explanation for phenotypic characteristics.

Table 3.4 Multidrug resistant rates for Pig *E. coli**

	Non-selective media		Selective media		All *E. coli*	
AM classes	# of R isolates	% of R isolates	# of R isolates	% of R isolates	# of R isolates	% of R isolates
0 (fully susceptible)	8	12%	0	0%	8	6%
1	5	8%	0	0%	5	4%
2	8	12%	2	3%	10	8%
3	13	20%	7	11%	20	16%
4	13	20%	4	7%	17	13%
5	13	20%	17	28%	30	24%
6	4	6%	20	33%	24	19%
7	1	2%	7	11%	8	6%
8	0	0%	4	7%	4	3%

*Note that this table excludes isolates that exceed NARMs cut off points for azithromycin and streptomycin (see Magiorakis et al. 2012)

Table 3.5 Multidrug resistant rates for Chicken *E. coli*

	Non-selective media				Selective media				All *E. coli*			
AM classes	# of R isolates		% of R isolates		# of R isolates		% of R isolates		# of R isolates		% of R isolates	
MIC plate	Old	New	Old	New	Old	New	Old	New	Old	New	Old	New
0 (fully susceptible)	3	4	19%	13%	0	0	0%	0%	3	4	10%	6%
1	1	1	6%	3%	0	0	0%	0%	1	1	3%	2%
2	0	3	0%	9%	0	1	0%	3%	0	4	0%	6%
3	5	3	31%	9%	1	2	7%	6%	6	5	20%	8%
4	4	3	25%	9%	2	4	14%	13%	6	7	20%	11%
5	3	8	19%	25%	2	6	14%	19%	5	14	17%	22%
6	0	9	0%	28%	6	8	43%	25%	6	17	20%	27%
7	0	1	0%	3%	3	10	21%	31%	3	11	10%	17%
8					0	1	0%	3%	0	1	0%	2%

3.2.3 *Salmonella*

Initial trials using cloacal swabs as the sample in 2018 yielded no *Salmonella* isolates from chickens. A shift to drag swabs was instituted in 2019 and this resulted in improved rates of isolation.

In total, 24 isolates were obtained from 17 chicken farms. The main serovars identified were *Salmonella* Infantis (5 from 4 farms), *S*. Weltveridin (7 from 6 farms),

S. Newport (3 from 2 farms), *S*. Agona (2 from 2 farms), *S*. Stanley (2 from 2 farms), *S*. Kentucky (1), *S*. 1,4,[5],12:i:- (1), *S*. Mbandaka (1) and 2 untyped Group E isolates.

Note that *Salmonella* Enteritidis was not detected. This is an important pathogen in southern China.

Twenty one isolates were obtained from 14 pig farms.

Serovars isolated from pigs included *Salmonella* 1,4,[5],12:i:- (monophasic variant strain of *Salmonella* Typhimurium) (5 from 4 farms). *S*. Rissen (4 from 3 farms), *S*. Altona (3 from 2 farms) *S*. London (3 from 2 farms), *S*. Stanley (2 from one farm), *S*. Weltveridin (2 from one farm), *S*. Typhimurium (1) and *S*. Bareilly (1).

Based on resistance patterns, 20 *Salmonella* isolates were selected and subjected to whole genome sequencing.

Salmonella isolates generally showed lower levels of resistance compared to *E. coli* isolates, especially those from chickens.

Only two isolates displayed resistance to 3rd generation cephalosporins (one *S*. Stanley and one *Salmonella* 1,4,[5],12:i:-) and low levels of resistance to fluoroquinolones were evident. The only chicken isolate that exceeded the breakpoint for fluoroquinolones had dual mutations in both *gyrA* and *parC*.

The organism from chickens carrying the greatest number of resistance genes (20) was a *Salmonella* 1,4,[5],12:i:- (see text box below). *Salmonella* Infantis from both pig and chicken contained $bla_{\text{CARB-2}}$.

No phenotypic resistance to carbapenems was detected.

Two chicken isolates carried *fosA7* genes but on testing these did not exceed fosfomycin breakpoints.

Salmonella 1,4,[5],12:i:- ST34

Salmonella 1,4,[5],12:i:- is a monophasic variant of *Salmonella* Typhimurium, that has increased in importance globally. It is now the most frequently detected multidrug resistant *Salmonella* serovar in the US. It can cause disease in pigs and has been associated with multiple outbreaks of foodborne illness in humans.

Six *S*. 1,4,[5],12:i:- sequence type 34 were detected on farms in 2019 from four pig farms and one chicken farm. Resistance patterns varied between the strains isolated but all were multidrug resistant.

The chicken isolate contained 20 resistance genes including *aac(3)-IId, aac(3)-IV, aac(6')-1aa, aadA2b, aadA, aph(3')-1a, aph3''-1b, aph(4)-1a, aph(6)-1d, bla*$_{\text{CTX-M-55}}$*, bla*$_{\text{OXA-10}}$*, lnuf, catA2, cmlA1, arr-2, sul1, sul3, dfrA14, tet(A)* and *qnrS1*. This isolate exceeded MIC breakpoints for gentamicin, streptomycin, ampicillin, 3rd and 4th generation cephalosporins, chloramphenicol/florfenicol, sulfonamide/trimethoprim and tetracyclines. It was non-wild type (NWT) for ciprofloxacin. The chicken farm on which this isolate was detected is not known to use antimicrobials and has no record of usage of 3rd generation cephalosporins.

One other pig isolate also contained the gene *bla*$_{\text{CTX-M-55}}$. Others have reported the presence of *bla*$_{\text{CTX-M-55}}$ in isolates from Cambodia (Nadminpalli et al. 2020).

Further surveillance will determine if these strains are persisting or present on other farms. Analysis will also be undertaken to see if there are any links between levels of heavy metals in feed and detection of this serovar (Bearson et al. 2020).

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Table 3.6 provides a summary of susceptibility test results for *Salmonella* isolates. Detailed results are available in Annex 1 Tables A1.7 to A1.8.

Table 3.6 Summary of AMR results for *Salmonella* from pigs and chickens[@]

Antimicrobial	Pig (n=21)	Chicken (n=24)	Notes
Amikacin**	ND	0%	
Amoxicillin/Clavulanic acid	0%	0%	
Ampicillin	57.1%	20.8%	
Azithromycin[@]	9.5%	0%	
Cefepime**	ND	5.9%	n=1/17 = *S.* 1,4,[5],12:i:-
Cefotaxime**	ND	5.9%	n=1/17 = *S.* 1,4,[5],12:i:-
Cefoxitin	0%	0%	
Ceftazidime**	ND	5.9%	n=1/17 = *S.* 1,4,[5],12:i:-
Ceftazidime/Avibactam*	0%	0%	
Ceftiofur	4.8%	4.2%	Pig *S.* Stanley, Chicken *S.* 1,4,[5],12:i:-
Ceftolozane/Tazobactam*	0%	0%	
Ceftriaxone	4.8%	4.2%	As above
Chloramphenicol	42.9%	16.7%	
Ciprofloxacin	4.8%	4.2%	NWT – pig 23.8%, chicken 20.8%
Colistin	0%	0%	
Florfenicol[@]**	ND	17.6%	
Fosfomycin**	ND	0%	
Gentamicin	19%	8.3%	
Imipenem**	ND	0%	
Levofloxacin**	ND	0%	NWT – chicken 17.6%
Meropenem	0%	0%	
Nalidixic acid	0%	12.5%	NWT – pig 9.5% chicken 16.7%
Nitrofurantoin**	ND	0%	
Piperacillin/Tazobactam	0%	0%	NWT – pig 4.8% chicken 8.3%
Streptomycin[@]	38.1%	25%	
Sulfisoxazole	47.6%	8.3%	
Tetracycline	71.4%	29.2%	
Tigecycline**	ND	0%	
Trimethoprim/Sulfamethoxazole	33.3%	16.7%	

*Due to introduction of a customised plate with an increased range of AMs during 2019 not all samples were tested against all AMs in this table. Those with a single asterisk were discontinued once the new customised plate was introduced.
**Those with two asterisks were added via the customised MIC plate.
@No CLSI or EUCAST clinical breakpoint. NARMS value used if available or EUCAST non-wild type.

3.2.4 *Enterococcus* spp.

Commensal *enterococci* have been included in a number of AMR monitoring programmes as a representative of Gram-positive organisms. They were included in the Hong Kong programme for pigs and chickens with a focus on *Enterococcus faecium* and *Enterococcus faecalis*.

Faecal specimens from pigs yielded 16 *E. faecium* isolates from 13 farms. Four *E. faecalis* isolates were obtained from three additional pig farms.

Chicken cloacal swabs yielded 23 *E. faecalis* isolates from 16 farms. No *E. faecium* were isolated from chicken samples.

Table 3.7 provides a summary of susceptibility test results for these organisms. Detailed results are available in Annex 1 Tables A1.9 to A1.11.

Table 3.7 Summary of AMR results for *Enterococcus* spp. from pigs and chickens

Antimicrobial	Pig *E. faecalis*	Chicken *E. faecalis*	Pig *E. faecium*	Notes
Ampicillin	0/4	0/23	0/16[1]	[1]1/16 NWT
Avilamycin	0/4[2]	0/23[2]	0/16[2]	[2]NWT
Chloramphenicol	1/4	6/23	1/16	
Ciprofloxacin	0/4	15/23	0/16	
Daptomycin	0/4	0/23[3]	1/16	[3]0/23 NWT
Erythromycin	1/4[4]	22/23	3/16	[4]1/4 NWT
Gentamicin (high dose)	1/4[5]	13/23[5]	0/16[5]	[5] High level resistance
Linezolid	0/4[6]	0/23[6]	0/16[6]	[6]1/4, 2/23 and 2/16 CLSI Intermediate
Nitrofurantoin	0/4[7]	0/23	2/16	[7]1/4 NWT
Quinupristin/dalfopristin	N/A[8]	N/A[8]	2/16	[8]Innate resistance
Streptomycin (high dose)	1/4[9]	16/23[9]	3/16[9]	[9]NWT
Tetracycline	3/4	21/23	8/16	
Tigecycline	0/4	1/23	0/16	
Vancomycin	0/4	0/23	0/16	

None of the isolates were resistant to vancomycin. Note that avoparcin was banned from being used in food animal production in Hong Kong since 2001.

Several isolates were categorised as intermediate "I" for linezolid (CLSI breakpoints).

A high proportion of chicken *E. faecalis* strains exceeded values for high level resistance to streptomycin and gentamicin. A high proportion of these isolates were resistant to erythromycin (see de Jong et al. 2019) and ciprofloxacin.

Two *E. faecium* isolates also exceeded EUCAST breakpoints for quinupristin/dalfopristin and another 14 were "I".

One *E. faecium* isolate from pigs exceeded CLSI breakpoints for daptomycin, consistent with findings from other countries (e.g. Lee et al. 2021).

Gene sequencing of seven isolates revealed the presence of a number of significant resistance genes including *poxtA* (2 pig *E. faecium*), *optrA* (1 pig *E. faecalis* that was phenotypically I for linezolid). Other genes linked to reduced susceptibility to aminoglycosides, erythromycin (*ermB* and *msrC*) and tetracycline (*tetM* and *tetL*) were also detected.

A number of AMR programmes elsewhere no longer include *Enterococcus* spp. in their testing programme. However, given the limited information available on these organisms in pigs and chickens in Hong Kong they have been included in the first years of the programme. The value of continuing this testing will be assessed as the programme proceeds.

3.2.5 *Campylobacter* spp.

Campylobacter spp. are carried by both pigs and chickens and are not recognised as primary pathogens in food animals.

In 2019, eight *Campylobacter coli* were isolated from pig faeces from seven farms. No *Campylobacter jejuni* were isolated from pigs and no *Campylobacter* spp. were isolated from chickens.

Results for susceptibility testing for these eight isolates are summarised in Table 3.8. Detailed results are provided in Annex 1 Table A1.12.

Table 3.8 Summary of AMR results for *Campylobacter coli* from pigs

Antimicrobial	Exceed CLSI breakpoint	Non-wild type (EUCAST)
Azithromycin	NA	3/8
Clindamycin	NA	4/8
Ciprofloxacin	8/8	8/8
Erythromycin	3/8	3/8
Florfenicol	NA	2/8
Gentamicin	NA	3/8
Nalidixic acid	NA	8/8
Tetracycline	8/8	8/8

Three of the isolates were multidrug resistant. One of these was non-wild type or resistant against all 8 AMs against which it was tested. The two other isolates were similar but were wild type for florfenicol (1 isolate) or gentamicin (1 isolate). Note that these three isolates belonged to different sequence types.

Quinolone resistance is widespread in *Campylobacter* spp. in Asia. It was detected in Hong Kong pig farms that are not known to be using fluoroquinolones. Persistence of fluoroquinolone resistance, even when this class of AMs is not being used, has been recognised elsewhere.

Two isolates were non-wild type for florfenicol. The gene *optrA,* which has been found in pig isolates in the mainland (Tang et al. 2020b), was detected in 5 of 8 isolates. These five isolates included the two that were non-wild type and all five had higher MICs against florfenicol than the three that did not possess this gene.

Three isolates were non-wild type for azithromycin and R for erythromycin (MIC=128 mg/L for both). These isolates had the A2075G mutation in 23S rRNA that is found in *C. coli* resistant to macrolides.

Although only a small number of isolates was obtained, the results suggest that, for severe cases of food-borne campylobacteriosis due to *C. coli,* resulting from handling contaminated carcasses/meat, it would be prudent to perform culture and susceptibility testing. Standard hygiene rules in food preparation should also be followed to prevent cross-contamination during food preparation.

The results are generally consistent with those seen in mainland China (e.g. Tang et al. 2020) and also in dogs and humans with multidrug resistant *C. jejuni* infections in

the US with the exception of decreased susceptibility to florfenicol that was detected in some isolates from Hong Kong SAR. The absence of *C. jejuni* in samples from chickens is an encouraging finding and possibly reflects differences in management practices for meat birds in Hong Kong compared to many other places.

3.2.6 Pathogens from pigs and chickens

Few samples were received for isolation of pathogens in clinical cases for AMR testing in 2019 by AFCD. During the trial period in 2018, four *Escherichia coli* isolates from diseased pigs were assessed by gene sequencing and phenotypic testing (using disc sensitivity). Two of these were from pigs with enteric disease and one was from a pig with severe meningoencephalitis likely secondary to concurrent porcine respiratory and reproductive syndrome virus infection. Genotypically they were found to carry genes encoding for resistance to 7 or 8 classes of AM (see Table 3.9).

The results demonstrate that few AMs would be available to treat these infections in pigs and the importance of gathering additional data on pathogens. This will allow appropriate guidance to be produced for local farmers in the face of disease outbreaks on the most appropriate AMs to use (and other measures to prevent and control bacterial infections). City University is continuing this work.

Note one of these isolates (O86:H11) belongs to a serotype recognised as an important cause of enterohaemorrhagic disease in humans. However a gene for production of shiga toxin was not detected.

Table 3.9 Resistance genes detected in *E. coli* isolates from sick and dead pigs

	Strain 1 ST410 O115:H28	Strain 2 ST1674 O11:H25	Strain 3 ST10 O27:H12	Strain 4 ST502 O86:H11 (Haemolytic)	Comment
Aminoglycoside	*aac(3)-IV* *aadA2* *aph(3')-Ia* *aph(3'')-Ib* *aph(4)-Ia* *aph(6)-Id*	*aadA2* *aph(3'')-Ib* *aph(6)-Id*	*aac(3)-IId* *aadA1* *aph(3')-Ia* *aph(3'')-Ib* *aph(6)-Id*	*aadA1* *aadA2* *ant(2'')-Ia* *aph(3')-Ia*	

Beta-lactam	*bla*$_{\text{CTX-M-65}}$ *bla*$_{\text{TEM-1B}}$	*bla*$_{\text{TEM-1A}}$	*bla*$_{\text{TEM-1B}}$	*bla*$_{\text{CMY-4}}$	
Colistin				*mcr-1.1*	Colistin gene chromosomal
Fluoroquinolone	*qnrS1* *gyrA* S83L D87N *parC* S80I *parE* S458A	*qnrS1*	*oqxA* *oqxB* *gyrA* S83L D87N *parC* S57T S80I	*oqxB* *gyrA* S83L D87N *parC* S57T S80I	
Macrolide, lincosamide, streptogramin B	*lnu(F)*		*mph(A)*		
Phenicols	*floR*	*floR*	*floR*	*catA1* *cmlA1*	
Sulphonamide, trimethoprim	*sul2* *dfrA17*	*sul2* *sul3* *dfrA12*	*sul1* *sul2* *dfrA1*	*sul3* *dfrA12*	
Tetracycline	*tet(B)* *tet(M)*	*tet(A)*	*tet(A)*	*tet(A)*	
Resistance genes – number of AM classes	*8*	*7*	*8*	*8*	

3.3 Links between usage and resistance

As described earlier correlations between usage of AMs and AMR patterns appear to be relatively weak, although only limited data are available. Because of limited data no statistical analysis has been conducted. Graphs and tables are provided as indications of possible trends.

The following comparisons were made.

Reported usage of fluoroquinolones and resistance in pig E. coli

Data from pig farms that do and do not report usage of fluoroquinolones were compared (Table 3.10). There was no apparent difference in the percentage resistant to ciprofloxacin.

Table 3.10 Comparison of susceptibility of commensal *E. coli* to fluoroquinolones between pig farms using and those not reporting usage of fluoroquinolones

	No report of usage (n=86)	Report of usage (n=40)
Ciprofloxacin %R	15.1%	17.5%
Ciprofloxacin %NWT	58.1%	57.5%

As discussed in the section on AM usage only very limited quantities of fluoroquinolones are reported as being used. Audit testing of faeces in 2019 was limited but will be expanded to detect possible unreported usage in future years.

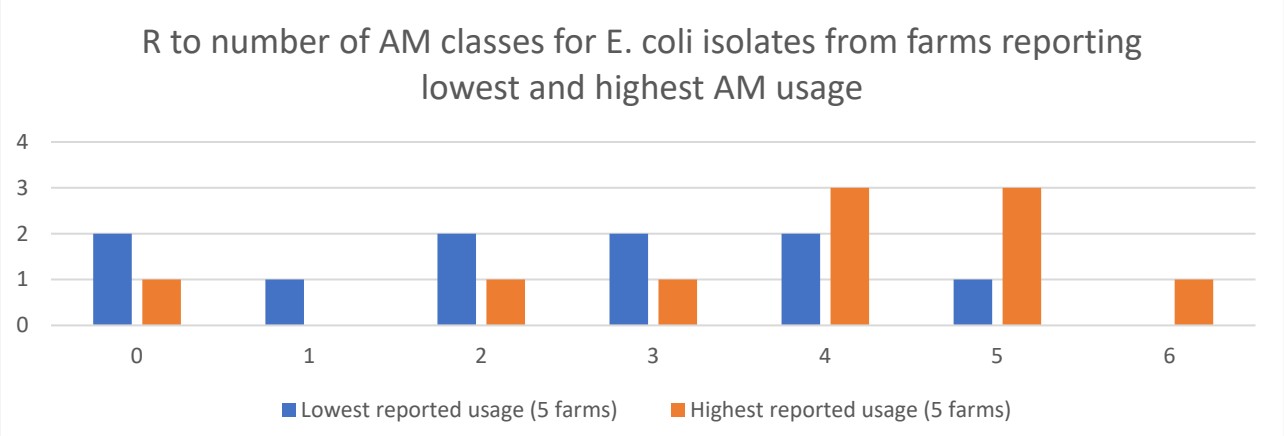
Overall usage vs resistance

Ten *E. coli* isolates (non-selective media) from the five pig farms with the lowest reported usage and ten *E. coli* isolates from the five farms with highest reported usage were compared for number of classes of AMs against which there was evidence of resistance or reduced susceptibility (Table 3.11).

Table 3.11 Comparison of multidrug resistance patterns between lowest and highest AMU pig farms

Number of AM classes	Lowest reported usage (5 farms)	Highest reported usage (5 farms)
0	2	1
1	1	0
2	2	1
3	2	1
4	2	3
5	1	3
6	0	1

Figure 3.1 Comparison of multidrug resistance patterns between lowest and highest AMU pig farms



Although only a small number of isolates were available for comparison, the results suggest that farms that report using greater quantities of AM have a higher number of multidrug resistant isolates, a higher maximum number of classes and also a higher median number of AM classes against which the organisms were non-susceptible. As more data are collected on AM usage and AMR patterns additional analysis will be conducted.

Nevertheless, it is evident that relationships between usage and resistance patterns are not always clear cut.

3.4 Studies on day-old chicks

Studies were commenced on day-old chicks to determine if resistance genes were being introduced to farms by day-old chicks. Thirty-five chicks were collected and pooled intestinal samples were cultured. From intestinal samples only two samples yielded *Escherichia coli* isolates and one of these also yielded *Klebsiella pneumoniae.* None of these isolates were non-susceptible to 3rd or 4th generation cephalosporins.

A trial was also conducted using metagenomics to identify resistance genes in 10 samples of intestine from birds from one batch. Although a number of resistance genes were detected these were not genes found in enterobacteria in birds at market weight. No genes encoding for resistance to 3rd and 4th generation cephalosporins or fluoroquinolones, the genes of most interest, were detected.

3.5 AMR in fish

3.5.1 Isolates obtained under the AMR surveillance programme

AMR studies in fish are usually based on pathogens isolated from clinical cases. However, few bacterial diseases are reported in pond and mariculture fish production in Hong Kong SAR, limiting the value of this method for monitoring resistance patterns. During trials in 2018 it was apparent that *Aeromonas* spp. (pond fish) and *Vibrio/Photobacterium* spp. (marine fish) could be cultured from swabs of scale mucus from a relatively high proportion of fish. It was also apparent, based on information from published research, that the skin of fish does have its own microbiome that can differ from that in the aquatic environment (see for example, Arias et al. 2013 and Zhang et al. 2019).

As a result of the likely limitations on clinical samples, swab samples from fish skin/skin mucus were collected for culture as the sample of choice for assessing AMR

in commensal organisms, on a trial basis. The organisms targeted as indicators were *Aeromonas* spp. for pond fish and *Vibrio* spp. and *Photobacterium damselae* for mariculture production. Some of these can be pathogenic for humans.

Note that there are few clinical break points or ECOFFs available for bacteria from fish. However, using a combination of MIC results and gene sequencing over time it is still possible to identify evidence of acquired resistance. Note that *Vibrio* spp. and *Aeromonas* spp. are intrinsically resistant to certain AMs. *Aeromonas* spp. can be identified based on their beta-lactamase gene phylogeny (Bertram et al. 2021).

In 2019, five *Aeromonas* spp. (identified by MALDI-TOF as *A. sobria* (4) and *A. hydrophila* (1)) were recovered. Among the observations were, three of five had what appeared to be high MIC values (>32) for ampicillin (see above re innate resistance) and one had what appears to be a high MIC value for fosfomycin (64).

A total of 22 *Vibrio* spp. isolates, predominantly identified as *V. alginolyticus,* were obtained. Sixteen of these had apparently high MIC values for colistin (16 isolates ≥16 mg/L); *mcr* genes were not detected in four isolates subjected to whole gene sequencing. Others have reported a number of pathways leading to apparent colistin resistance that do not involve transmissible *mcr* genes. Some of these are reversible through modification of the bacterial metabolome (Li et al. 2020).

Seventeen of the 22 *Vibrio* spp. isolates were apparently R for ampicillin (MIC≥32), in line with findings from elsewhere. Three were also R for cefoxitin. In four isolates, from 2019, that were sequenced, no beta-lactamase resistance genes were detected. Other chromosomal resistance genes detected were *tet34 and mdtK.* No apparent reduced susceptibility to tetracyclines or ciprofloxacin were detected in any *Vibrio* spp. isolates, despite the presence of these genes.

Twenty-five *Photobacterium* spp. were grown and were generally regarded as susceptible to all AM classes, with the possible exception of sulphonamides (MIC to sulfisoxazole ≥128 in 24/25 isolates). Several isolates appear to have reduced susceptibility to ampicillin (1/25 with MIC=32) and colistin (2/25 with MIC=16). Gene sequencing of one isolate from 2019 revealed no known resistance genes.

AFCD will continue to assess the usefulness of testing of commensal bacteria from skin mucus over the next few years.

MIC values for isolates from fish are provided in Annex 1 Tables A1.13 to A1.15.

3.5.2 Isolates obtained from fish diagnostic specimens

Information was obtained on AM susceptibility testing conducted on organisms isolated from diseased fish. The organisms (predominantly *Aeromonas* spp. and *Vibrio* spp.) were tested using disk diffusion methods against a limited range of AMs.

Among the findings of note were two of 16 *Vibrio* spp. and one of nine *Aeromonas* spp. that were reported as being R to florfenicol. Five of the 16 *Vibrio* spp. (including the two that were reported as R for florfenicol) were reported as being R to oxytetracycline. Four of the nine *Aeromonas* spp. were also reported as being R to oxytetracycline, including the one isolate that was reported as R to florfenicol. These two AMs were the only drugs used in treatment of fish diseases in 2019. No gene sequencing results are available for these organisms to assess links between phenotype and genotype. It is anticipated that additional studies will be conducted on pathogens collected in the future.

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Annex 1

Table A1.1 MIC distribution of pig *E. coli* isolated from non-selective media

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																Break point	Susceptibility			ECOFF		
		0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Amoxicillin/clavulanic acid	65							2	4	13	28	14	3	1				CLSI	47	14	4	8	47	18
	100							3.1	6.2	20.0	43.1	21.5	4.6	1.5				32	72.3	21.5	6.2		72.3	27.7
Ampicillin	65							2	10	11	0	0	1	41				CLSI	23	0	42	8	23	42
	100							3.1	15.4	16.9	0.0	0.0	1.5	63.1				32	35.4	0.0	64.6		35.4	64.6
Azithromycin	65				0	1	0	0	9	38	14	1	2					NARMS	63	0	2	16	63	2
	100				0.0	1.5	0.0	0.0	13.8	58.5	21.5	1.5	3.1					32	96.9	0.0	3.1		96.9	3.1
Cefoxitin	65						1	1	6	34	17	4	1	1				CLSI	59	4	2	8	59	6
	100						1.5	1.5	9.2	52.3	26.2	6.2	1.5	1.5				32	90.8	6.2	3.1		90.8	9.2
Ceftazidime/avibactam	65							65	0	0	0	0						CLSI	65	0	0	N/A		
	100							100.0	0.0	0.0	0.0	0.0						16	100.0	0.0	0.0			
Ceftiofur	65				1	17	38	4	1	3	1	0						NARMS	61	3	1	1	60	5
	100				1.5	26.2	58.5	6.2	1.5	4.6	1.5	0.0						8	93.8	4.6	1.5		92.3	7.7
Ceftolozane/tazobactam	65					61	4	0	0	0	0							CLSI	65	0	0	1	65	0
	100					93.8	6.2	0.0	0.0	0.0	0.0							8	100.0	0.0	0.0		100.0	0.0
Ceftriaxone	65					58	1	1	0	3	0	2	0	0	0			CLSI	60	0	5	0.125	N/A	N/A
	100					89.2	1.5	1.5	0.0	4.6	0.0	3.1	0.0	0.0	0.0			4	92.3	0.0	7.7			
Chloramphenicol	65								0	7	12	5	3	38				CLSI	19	5	41	16	24	41
	100								0.0	10.8	18.5	7.7	4.6	58.5				32	29.2	7.7	63.1		36.9	63.1
Ciprofloxacin	65	27	2	2	6	15	7	2	0	1	3							CLSI	52	7	6	0.064	31	34
	100	41.5	3.1	3.1	9.2	23.1	10.8	3.1	0.0	1.5	4.6							1	80.0	10.8	9.2		47.7	52.3
Colistin	65					0	24	38	2	1	0							EUCAST	64	0	1	2	64	1
	100					0.0	36.9	58.5	3.1	1.5	0.0							4	98.5	0.0	1.5		98.5	1.5
Gentamicin	65					0	21	32	5	0	0	1	6					CLSI	58	0	7	2	58	7
	100					0.0	32.3	49.2	7.7	0.0	0.0	1.5	9.2					16	89.2	0.0	10.8		89.2	10.8
Meropenem	65			6	59	0	0	0	0	0	0	0						CLSI	65	0	0	0.125	65	0
	100			9.2	90.8	0.0	0.0	0.0	0	0	0	0						4	100.0	0.0	0.0		100.0	0.0
Nalidixic acid	65						0	0	21	15	9	3	2	15				NARMS	48	0	17	8	45	20
	100						0.0	0.0	32.3	23.1	13.8	4.6	3.1	23.1				32	73.8	0.0	26.2		69.2	30.8
Piperacillin/tazobactam	65							20	37	7	1	0	0					CLSI	65	0	0	8	65	0
	100							30.8	56.9	10.8	1.5	0.0	0.0					128	100.0	0.0	0.0		100.0	0.0
Streptomycin	65								0	5	14	10	4	14	18			NARMS	29	0	36	16	29	36
	100								0.0	7.7	21.5	15.4	6.2	21.5	27.7			32	44.6	0.0	55.4		44.6	55.4
Sulfisoxazole	65											26	1	0	0	0	38	CLSI	27	0	38	N/A		
	100											40.0	1.5	0.0	0.0	0.0	58.5	512	41.5	0.0	58.5			
Tetracycline	65									14	1	3	10	37				CLSI	14	1	50	8	15	50
	100									21.5	1.5	4.6	15.4	56.9				16	21.5	1.5	76.9		23.1	76.9
Trimethoprim/sulfamethoxazole	65				23	7	5	2	0	1	27							CLSI	37	0	28	0.25	30	35
	100				35.4	10.8	7.7	3.1	0.0	1.5	41.5							4	56.9	0.0	43.1		46.2	53.8

Table A1.2 MIC distribution of pig *E. coli* isolated from selective media

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																Break point	Susceptibility			ECOFF		
		0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Amoxicillin/clavulanic acid	61							0	0	6	31	13	7	4				CLSI	37	13	11	8	37	24
	100							0.0	0.0	9.8	50.8	21.3	11.5	6.6				32	60.7	21.3	18.0		60.7	39.3
Ampicillin	61							0	0	0	0	0	0	61				CLSI	0	0	61	8	0	61
	100							0.0	0.0	0.0	0.0	0.0	0.0	100.0				32	0.0	0.0	100.0		0.0	100.0
Azithromycin	61				0	0	0	0	7	30	12	3	9					NARMS	52	0	9	16	52	9
	100				0.0	0.0	0.0	0.0	11.5	49.2	19.7	4.9	14.8					32	85.2	0.0	14.8		85.2	14.8
Cefoxitin	61						0	0	9	27	12	4	5	4				CLSI	48	4	9	8	48	13
	100						0.0	0.0	14.8	44.3	19.7	6.6	8.2	6.6				32	78.7	6.6	14.8		78.7	21.3
Ceftazidime/avibactam	61							60	0	1	0	0						CLSI	61	0	0	N/A		
	100							98.4	0.0	1.6	0.0	0.0						16	100.0	0.0	0.0			
Ceftiofur	61				0	0	0	0	0	6	6	49						NARMS	0	6	55	1	0	61
	100				0.0	0.0	0.0	0.0	0.0	9.8	9.8	80.3						8	0.0	9.8	90.2		0.0	100.0
Ceftolozane/tazobactam	61					39	19	3	0	0	0							CLSI	61	0	0	1	61	0
	100					63.9	31.1	4.9	0.0	0.0	0.0							8	100.0	0.0	0.0		100.0	0.0
Ceftriaxone	61					0	0	0	0	4	6	8	15	13	15			CLSI	0	0	61	0.125	N/A	N/A
	100					0.0	0.0	0.0	0.0	6.6	9.8	13.1	24.6	21.3	24.6			4	0.0	0.0	100.0			
Chloramphenicol	61								0	3	10	3	3	42				CLSI	13	3	45	16	16	45
	100								0.0	4.9	16.4	4.9	4.9	68.9				32	21.3	4.9	73.8		26.2	73.8
Ciprofloxacin	61	16	2	4	8	12	5	2	2	3	7							CLSI	42	5	14	0.064	22	39
	100	26.2	3.3	6.6	13.1	19.7	8.2	3.3	3.3	4.9	11.5							1	68.9	8.2	23.0		36.1	63.9
Colistin	61					0	35	26	0	0	0							EUCAST	61	0	0	2	61	0
	100					0.0	57.4	42.6	0.0	0.0	0.0							4	100.0	0.0	0.0		100.0	0.0
Gentamicin	61					0	15	28	5	0	0	0	13					CLSI	48	0	13	2	48	13
	100					0.0	24.6	45.9	8.2	0.0	0.0	0.0	21.3					16	78.7	0.0	21.3		78.7	21.3
Meropenem	61			0	60	1	0	0	0	0	0	0						CLSI	61	0	0	0.125	60	1
	100			0.0	98.4	1.6	0.0	0.0	0	0	0	0						4	100.0	0.0	0.0		98.4	1.6
Nalidixic acid	61						0	1	16	12	5	2	1	24				NARMS	36	0	25	8	34	27
	100						0.0	1.6	26.2	19.7	8.2	3.3	1.6	39.3				32	59.0	0.0	41.0		55.7	44.3
Piperacillin/tazobactam	61							7	44	9	1	0	0					CLSI	61	0	0	8	61	0
	100							11.5	72.1	14.8	1.6	0.0	0.0					128	100.0	0.0	0.0		100.0	0.0
Streptomycin	61								0	1	9	8	6	4	33			NARMS	18	0	43	16	18	43
	100								0.0	1.6	14.8	13.1	9.8	6.6	54.1			32	29.5	0.0	70.5		29.5	70.5
Sulfisoxazole	61											13	0	0	0	0	48	CLSI	13	0	48	N/A		
	100											21.3	0.0	0.0	0.0	0.0	78.7	512	21.3	0.0	78.7			
Tetracycline	61									8	0	1	11	41				CLSI	8	0	53	8	8	53
	100									13.1	0.0	1.6	18.0	67.2				16	13.1	0.0	86.9		13.1	86.9
Trimethoprim/sulfamethoxazole	61				15	8	4	0	1	1	32							CLSI	28	0	33	0.25	23	38
	100				24.6	13.1	6.6	0.0	1.6	1.6	52.5							4	45.9	0.0	54.1		37.7	62.3

Table A1.3 MIC distribution of all pig *E. coli* (including those isolated from selective media)

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																Break point	Susceptibility			ECOFF		
		0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Amoxicillin/clavulanic acid	126							2	4	19	59	27	10	5				CLSI	84	27	15	8	84	42
	100							1.6	3.2	15.1	46.8	21.4	7.9	4.0				32	66.7	21.4	11.9		66.7	33.3
Ampicillin	126							2	10	11	0	0	1	102				CLSI	23	0	103	8	23	103
	100							1.6	7.9	8.7	0.0	0.0	0.8	81.0				32	18.3	0.0	81.7		18.3	81.7
Azithromycin	126				0	1	0	0	16	68	26	4	11					NARMS	115	0	11	16	115	11
	100				0.0	0.8	0.0	0.0	12.7	54.0	20.6	3.2	8.7					32	91.3	0.0	8.7		91.3	8.7
Cefoxitin	126						1	1	15	61	29	8	6	5				CLSI	107	8	11	8	107	19
	100						0.8	0.8	11.9	48.4	23.0	6.3	4.8	4.0				32	84.9	6.3	8.7		84.9	15.1
Ceftazidime/avibactam	126							125	0	1	0	0						CLSI	126	0	0	N/A		
	100							99.2	0.0	0.8	0.0	0.0						16	100.0	0.0	0.0			
Ceftiofur	126				1	17	38	4	1	9	7	49						NARMS	61	9	56	1	60	66
	100				0.8	13.5	30.2	3.2	0.8	7.1	5.6	38.9						8	48.4	7.1	44.4		47.6	52.4
Ceftolozane/tazobactam	126					100	23	3	0	0	0							CLSI	126	0	0	1	126	0
	100					79.4	18.3	2.4	0.0	0.0	0.0							8	100.0	0.0	0.0		100.0	0.0
Ceftriaxone	126					58	1	1	0	7	6	10	15	13	15			CLSI	60	0	66	0.125	N/A	N/A
	100					46.0	0.8	0.8	0.0	5.6	4.8	7.9	11.9	10.3	11.9			4	47.6	0.0	52.4			
Chloramphenicol	126								0	10	22	8	6	80				CLSI	32	8	86	16	40	86
	100								0.0	7.9	17.5	6.3	4.8	63.5				32	25.4	6.3	68.3		31.7	68.3
Ciprofloxacin	126	43	4	6	14	27	12	4	2	4	10							CLSI	94	12	20	0.064	53	73
	100	34.1	3.2	4.8	11.1	21.4	9.5	3.2	1.6	3.2	7.9							1	74.6	9.5	15.9		42.1	57.9
Colistin	126					0	59	64	2	1	0							EUCAST	125	0	1	2	125	1
	100					0.0	46.8	50.8	1.6	0.8	0.0							4	99.2	0.0	0.8		99.2	0.8
Gentamicin	126					0	36	60	10	0	0	1	19					CLSI	106	0	20	2	106	20
	100					0.0	28.6	47.6	7.9	0.0	0.0	0.8	15.1					16	84.1	0.0	15.9		84.1	15.9
Meropenem	126			6	119	1	0	0	0	0	0	0						CLSI	126	0	0	0.125	125	1
	100			4.8	94.4	0.8	0.0	0.0	0	0	0	0						4	100.0	0.0	0.0		99.2	0.8
Nalidixic acid	126						0	1	37	27	14	5	3	39				NARMS	84	0	42	8	79	47
	100						0.0	0.8	29.4	21.4	11.1	4.0	2.4	31.0				32	66.7	0.0	33.3		62.7	37.3
Piperacillin/tazobactam	126							27	81	16	2	0	0					CLSI	126	0	0	8	126	0
	100							21.4	64.3	12.7	1.6	0.0	0.0					128	100.0	0.0	0.0		100.0	0.0
Streptomycin	126								0	6	23	18	10	18	51			NARMS	47	0	79	16	47	79
	100								0.0	4.8	18.3	14.3	7.9	14.3	40.5			32	37.3	0.0	62.7		37.3	62.7
Sulfisoxazole	126											39	1	0	0	0	86	CLSI	40	0	86	N/A		
	100											31.0	0.8	0.0	0.0	0.0	68.3	512	31.7	0.0	68.3			
Tetracycline	126									22	1	4	21	78				CLSI	22	1	103	8	23	103
	100									17.5	0.8	3.2	16.7	61.9				16	17.5	0.8	81.7		18.3	81.7
Trimethoprim/sulfamethoxazole	126				38	15	9	2	1	2	59							CLSI	65	0	61	0.25	53	73
	100				30.2	11.9	7.1	1.6	0.8	1.6	46.8							4	51.6	0.0	48.4		42.1	57.9

Table A1.4 MIC distribution of chicken *E. coli* isolated from non-selective media (1/2)

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																	Break point	Susceptibility			ECOFF		
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Amikacin	32								0	26	6	0	0	0	0				CLSI	32	0	0	8	32	0
	100								0.0	81.3	18.8	0.0	0.0	0.0	0.0				64	100.0	0.0	0.0		100.0	0.0
Amoxicillin/clavulanic acid	48								0	3	17	26	2	0	0				CLSI	46	2	0	8	46	2
	100								0.0	6.3	35.4	54.2	4.2	0.0	0.0				32	95.8	4.2	0.0		95.8	4.2
Ampicillin	48								2	4	6	1	1	1	33				CLSI	13	1	34	8	13	35
	100								4.2	8.3	12.5	2.1	2.1	2.1	68.8				32	27.1	2.1	70.8		27.1	72.9
Azithromycin	48						0	0	3	19	22	4	0	0	0				NARMS	48	0	0	16	48	0
	100						0.0	0.0	0.0	6.3	35.4	54.2	4.2	0.0	0.0				32	100.0	0.0	0.0		100.0	0.0
Cefepime	32				21	3	0	0	0	1	6	0	1	0					CLSI	25	6	1	0.25	24	8
	100				65.6	9.4	0.0	0.0	0.0	3.1	18.8	0.0	3.1	0.0					16	78.1	18.8	3.1		75.0	25.0
Cefotaxime	32						22	1	0	1	1	7							CLSI	23	1	8	0.25	22	10
	100						68.8	3.1	0.0	3.1	3.1	21.9							4	71.9	3.1	25.0		68.8	31.3
Cefoxitin	48							0	1	11	29	6	1	0	0				CLSI	47	1	0	8	47	1
	100							0.0	2.1	22.9	60.4	12.5	2.1	0.0	0.0				32	97.9	2.1	0.0		97.9	2.1
Ceftazidime	32							24	1	0	6	1	0						CLSI	31	1	0	0.5	24	8
	100							75.0	3.1	0.0	18.8	3.1	0.0						16	96.9	3.1	0.0		75.0	25.0
Ceftazidime/avibactam	16								16	0	0	0	0						CLSI	16	0	0	N/A		
	100								100.0	0.0	0.0	0.0	0.0						16	100.0	0.0	0.0			
Ceftiofur	48					2	14	17	3	1	0	0	11						NARMS	37	0	11	1	36	12
	100					4.2	29.2	35.4	6.3	2.1	0.0	0.0	22.9						8	77.1	0.0	22.9		75.0	25.0
Ceftolozane/tazobactam	16						15	1	0	0	0	0							CLSI	16	0	0	1	16	0
	100						93.8	6.3	0.0	0.0	0.0	0.0							8	100.0	0.0	0.0		100.0	0.0
Ceftriaxone	48						37	2	0	0	0	0	0	4	2	3			CLSI	39	0	9	0.125	0	48
	100						77.1	4.2	0.0	0.0	0.0	0.0	0.0	8.3	4.2	6.3			4	81.3	0.0	18.8		0.0	100.0
Chloramphenicol	48									5	9	7	3	5	19				CLSI	21	3	24	16	24	24
	100									10.4	18.8	14.6	6.3	10.4	39.6				32	43.8	6.3	50.0		50.0	50.0
Ciprofloxacin	48		13	1	5	5	11	0	1	0	3	9							CLSI	35	0	13	0.064	19	29
	100		27.1	2.1	10.4	10.4	22.9	0.0	2.1	0.0	6.3	18.8							1	72.9	0.0	27.1		39.6	60.4
Colistin	48						0	11	37	0	0	0	0						EUCAST	37	0	0	2	37	0
	100						0.0	22.9	77.1	0.0	0.0	0.0	0.0						4	77.1	0.0	0.0		77.1	0.0

Table A1.4 MIC distribution of chicken *E. coli* isolated from non-selective media (2/2)

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																	Break point	Susceptibility			ECOFF		
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Florfenicol	32 100								2 6.3	5 15.6	10 31.3	2 6.3	2 6.3	5 15.6	6 18.8				**N/A**				**16**	21 65.6	11 34.4
Fosfomycin	32 100								22 68.8	7 21.9	3 9.4	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0			**EUCAST 64**	32 100.0	0 0.0	0 0.0	**4**	32 100.0	0 0.0
Gentamicin	48 100						1 2.1	12 25.0	12 25.0	2 4.2	0 0.0	7 14.6	5 10.4	9 18.8					**CLSI 16**	27 56.3	7 14.6	14 29.2	**2**	27 56.3	21 43.8
Imipenem	32 100					19 59.4	13 40.6	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0					**CLSI 4**	32 100.0	0 0.0	0 0.0	**0.5**	32 100.0	0 0.0
Levofloxacin	32 100						14 43.8	4 12.5	1 3.1	2 6.3	3 9.4	8 25.0							**CLSI 2**	18 56.3	1 3.1	13 40.6	**0.25**	14 43.8	18 56.3
Meropenem	48 100				32 66.7	16 33.3	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0								**CLSI 4**	48 100.0	0 0.0	0 0.0	**0.125**	48 100.0	0 0.0
Nalidixic acid	48 100							0 0.0	1 2.1	9 18.8	15 31.3	2 4.2	3 6.3	3 6.3	15 31.3				**CLSI 32**	30 62.5	0 0.0	18 37.5	**8**	27 56.3	21 43.8
Nitrofurantoin	32 100												29 90.6	3 9.4	0 0.0	0 0.0			**CLSI 128**	32 100.0	0 0.0	0 0.0	**64**	32 100.0	0 0.0
Oxolinic acid	32 100	0 0.0	0 0.0	0 0.0	0 0.0	1 3.1	6 18.8	25 78.1											**N/A**				**N/A**		
Piperacillin/tazobactam	48 100								27 56.3	19 39.6	2 4.2	0 0.0	0 0.0	0 0					**CLSI 128**	48 100.0	0 0.0	0 0.0	**8**	48 100.0	0 0.0
Streptomycin	48 100									0 0.0	1 2.1	10 20.8	6 12.5	4 8.3	5 10.4	22 45.8			**NARMS 32**	17 35.4	0 0.0	31 64.6	**16**	17 35.4	31 64.6
Sulfisoxazole	48 100												12 25.0	0 0.0	0 0.0	2 4.2	1 2.1	33 68.8	**CLSI 512**	15 31.3	0 0.0	33 68.8	**N/A**		
Temocillin	32 100								1 3.1	7 21.9	14 43.8	10 31.3	0 0						**EUCAST 32**	0 0.0	32 100.0	0 0.0	**16**	32 100.0	0 0.0
Tetracycline	48 100										8 16.7	3 6.3	4 8.3	16 33.3	17 35.4				**CLSI 16**	8 16.7	3 6.3	37 77.1	**8**	11 22.9	37 77.1
Tigecycline	32 100						25 78.1	6 18.8	1 3.1	0 0.0	0 0.0	0 0.0							**EUCAST 1**	31 96.9	0 0.0	1 3.1	**0.5**	31 96.9	1 3.1
Trimethoprim/sulfamethoxazole	48 100					15 31.3	2 4.2	2 4.2	1 2.1	1 2.1	0 0.0	27 56.3							**CLSI 4**	21 43.8	0 0.0	27 56.3	**0.25**	17 35.4	31 64.6

Table A1.5 MIC distribution of chicken *E. coli* isolated from selective media (1/2)

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																	Break point	Susceptibility			ECOFF		
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Amikacin	32								3	22	4	2	1	0	0				CLSI	32	0	0	8	31	1
	100								9.4	68.8	12.5	6.3	3.1	0.0	0.0				64	100.0	0.0	0.0		96.9	3.1
Amoxicillin/clavulanic acid	46								0	1	7	30	7	0	1				CLSI	38	7	1	8	38	8
	100								0.0	2.2	15.2	65.2	15.2	0.0	2.2				32	82.6	15.2	2.2		82.6	17.4
Ampicillin	46								0	0	0	0	0	0	46				CLSI	0	0	46	8	0	46
	100								0.0	0.0	0.0	0.0	0.0	0.0	100.0				32	0.0	0.0	100.0		0.0	100.0
Azithromycin	46						0	0	2	19	19	3	2	0	1				NARMS	45	0	1	16	45	1
	100						0.0	0.0	0.0	2.2	15.2	65.2	15.2	0.0	2.2				32	97.8	0.0	2.2		97.8	2.2
Cefepime	32				1	1	0	2	2	6	12	2	4	2					CLSI	12	14	6	0.25	2	30
	100				3.1	3.1	0.0	6.3	6.3	18.8	37.5	6.3	12.5	6.3					16	37.5	43.8	18.8		6.3	93.8
Cefotaxime	32						2	0	0	1	1	28							CLSI	2	1	29	0.25	2	30
	100						6.3	0.0	0.0	3.1	3.1	87.5							4	6.3	3.1	90.6		6.3	93.8
Cefoxitin	46							0	0	7	20	15	3	0	1				CLSI	42	3	1	8	42	3
	100							0.0	0.0	15.2	43.5	32.6	6.5	0.0	2.2				32	91.3	6.5	2.2		91.3	6.5
Ceftazidime	32							7	2	5	5	7	6						CLSI	19	7	6	0.5	7	25
	100							21.9	6.3	15.6	15.6	21.9	18.8						16	59.4	21.9	18.8		21.9	78.1
Ceftazidime/avibactam	14								14	0	0	0	0						CLSI	14	0	0	N/A		
	100								100.0	0.0	0.0	0.0	0.0						16	100.0	0.0	0.0			
Ceftiofur	46					0	1	1	0	1	0	2	41						NARMS	3	0	43	1	2	44
	100					0.0	2.2	2.2	0.0	2.2	0.0	4.3	89.1						8	6.5	0.0	93.5		4.3	95.7
Ceftolozane/tazobactam	14						11	3	0	0	0	0							CLSI	14	0	0	1	14	0
	100						78.6	21.4	0.0	0.0	0.0	0.0							8	100.0	0.0	0.0		100.0	0.0
Ceftriaxone	46						2	0	0	0	1	0	4	5	10	24			CLSI	2	0	44	0.125	0	46
	100						4.3	0.0	0.0	0.0	2.2	0.0	8.7	10.9	21.7	52.2			4	4.3	0.0	95.7		0.0	100.0
Chloramphenicol	46									0	6	5	1	3	31				CLSI	11	1	34	16	12	34
	100									0.0	13.0	10.9	2.2	6.5	67.4				32	23.9	2.2	73.9		26.1	73.9
Ciprofloxacin	46		2	0	0	4	11	6	2	2	3	16							CLSI	17	6	23	0.064	2	44
	100		4.3	0.0	0.0	8.7	23.9	13.0	4.3	4.3	6.5	34.8							1	37.0	13.0	50.0		4.3	95.7
Colistin	46						1	12	30	1	1	1	0						EUCAST	31	0	2	2	31	2
	100						2.2	26.1	65.2	2.2	2.2	2.2	0.0						4	67.4	0.0	4.3		67.4	4.3

Table A1.5 MIC distribution of chicken *E. coli* isolated from selective media (2/2)

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																	Break point	Susceptibility			ECOFF		
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Florfenicol	32 100								0 0.0	3 9.4	3 9.4	3 9.4	3 9.4	2 6.3	18 56.3				N/A				16	12 37.5	20 62.5
Fosfomycin	32 100								17 53.1	6 18.8	0 0.0	1 3.1	1 3.1	1 3.1	1 3.1	5 15.6			EUCAST 64	26 81.3	0 0.0	6 18.8	4	23 71.9	9 28.1
Gentamicin	46 100						0 0.0	10 21.7	9 19.6	1 2.2	2 4.3	8 17.4	8 17.4	8 17.4					CLSI 16	22 47.8	8 17.4	16 34.8	2	20 43.5	26 56.5
Imipenem	32 100					14 43.8	18 56.3	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0					CLSI 4	32 100.0	0 0.0	0 0.0	0.5	32 100.0	0 0.0
Levofloxacin	32 100						6 18.8	4 12.5	3 9.4	2 6.3	6 18.8	11 34.4							CLSI 2	10 31.3	3 9.4	19 59.4	0.25	6 18.8	26 81.3
Meropenem	46 100				32 69.6	14 30.4	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0								CLSI 4	46 100.0	0 0.0	0 0.0	0.125	46 100.0	0 0.0
Nalidixic acid	46 100							0 0.0	0 0.0	6 13.0	2 4.3	2 4.3	1 2.2	0 0.0	35 76.1				CLSI 32	11 23.9	0 0.0	35 76.1	8	10 21.7	36 78.3
Nitrofurantoin	32 100												29 90.6	3 9.4	0 0.0	0 0.0			CLSI 128	32 100.0	0 0.0	0 0.0	64	32 100.0	0 0.0
Oxolinic acid	32 100	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	1 3.1	31 96.9											N/A				N/A		
Piperacillin/tazobactam	46 100								14 30.4	23 50.0	4 8.7	5 10.9	0 0.0	0 0					CLSI 128	46 100.0	0 0.0	0 0.0	8	46 100.0	0 0.0
Streptomycin	46 100									0 0.0	0 0.0	3 6.5	2 4.3	3 6.5	6 13.0	32 69.6			NARMS 32	5 10.9	0 0.0	41 89.1	16	5 10.9	41 89.1
Sulfisoxazole	46 100												8 17.4	3 6.5	0 0.0	0 0.0	1 2.2	34 73.9	CLSI 512	12 26.1	0 0.0	34 73.9	N/A		
Temocillin	32 100								0 0.0	3 9.4	17 53.1	10 31.3	2 6.25						EUCAST 32	0 0.0	32 100.0	0 0.0	16	32 100.0	0 0.0
Tetracycline	46 100										5 10.9	2 4.3	12 26.1	10 21.7	17 37.0				CLSI 16	5 10.9	2 4.3	39 84.8	8	7 15.2	39 84.8
Tigecycline	32 100						25 78.1	7 21.9	0 0.0	0 0.0	0 0.0	0 0.0							EUCAST 1	32 100.0	0 0.0	0 0.0	0.5	32 100.0	0 0.0
Trimethoprim/sulfamethoxazole	46 100					11 23.9	6 13.0	2 4.3	2 4.3	0 0.0	1 2.2	24 52.2							CLSI 4	21 45.7	0 0.0	25 54.3	0.25	17 37.0	29 63.0

Table A1.6 MIC distribution of all chicken *E. coli* (including those isolated from selective media) (1/2)

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																	Break point	Susceptibility			ECOFF		
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Amikacin	64								3	48	10	2	1	0	0				CLSI	64	0	0	8	63	1
	100								4.7	75.0	15.6	3.1	1.6	0.0	0.0				64	100.0	0.0	0.0		98.4	1.6
Amoxicillin/clavulanic acid	94								0	4	24	56	9	0	1				CLSI	84	9	1	8	84	10
	100								0.0	4.3	25.5	59.6	9.6	0.0	1.1				32	89.4	9.6	1.1		89.4	10.6
Ampicillin	94								2	4	6	1	1	1	79				CLSI	13	1	80	8	13	81
	100								2.1	4.3	6.4	1.1	1.1	1.1	84.0				32	13.8	1.1	85.1		13.8	86.2
Azithromycin	94						0	0	5	38	41	7	2	0	1				NARMS	93	0	1	16	93	1
	100						0.0	0.0	0.0	4.3	25.5	59.6	9.6	0.0	1.1				32	98.9	0.0	1.1		98.9	1.1
Cefepime	64				22	4	0	2	2	7	18	2	5	2					CLSI	37	20	7	0.25	26	38
	100				34.4	6.3	0.0	3.1	3.1	10.9	28.1	3.1	7.8	3.1					16	57.8	31.3	10.9		40.6	59.4
Cefotaxime	64						24	1	0	2	2	35							CLSI	25	2	37	0.25	24	40
	100						37.5	1.6	0.0	3.1	3.1	54.7							4	39.1	3.1	57.8		37.5	62.5
Cefoxitin	94							0	1	18	49	21	4	0	1				CLSI	89	4	1	8	89	4
	100							0.0	1.1	19.1	52.1	22.3	4.3	0.0	1.1				32	94.7	4.3	1.1		94.7	4.3
Ceftazidime	64							31	3	5	11	8	6						CLSI	50	8	6	0.5	31	33
	100							48.4	4.7	7.8	17.2	12.5	9.4						16	78.1	12.5	9.4		48.4	51.6
Ceftazidime/avibactam	30								30	0	0	0	0						CLSI	30	0	0	N/A		
	100								100.0	0.0	0.0	0.0	0.0						16	100.0	0.0	0.0			
Ceftiofur	94					2	15	18	3	2	0	2	52						NARMS	40	0	54	1	38	56
	100					2.1	16.0	19.1	3.2	2.1	0.0	2.1	55.3						8	42.6	0.0	57.4		40.4	59.6
Ceftolozane/tazobactam	30						26	4	0	0	0	0							CLSI	30	0	0	1	30	0
	100						86.7	13.3	0.0	0.0	0.0	0.0							8	100.0	0.0	0.0		100.0	0.0
Ceftriaxone	94						39	2	0	0	1	0	4	9	12	27			CLSI	41	0	53	0.125	0	94
	100						41.5	2.1	0.0	0.0	1.1	0.0	4.3	9.6	12.8	28.7			4	43.6	0.0	56.4		0.0	100.0
Chloramphenicol	94									5	15	12	4	8	50				CLSI	32	4	58	16	36	58
	100									5.3	16.0	12.8	4.3	8.5	53.2				32	34.0	4.3	61.7		38.3	61.7
Ciprofloxacin	94		15	1	5	9	22	6	3	2	6	25							CLSI	52	6	36	0.064	21	73
	100		16.0	1.1	5.3	9.6	23.4	6.4	3.2	2.1	6.4	26.6							1	55.3	6.4	38.3		22.3	77.7
Colistin	94						1	23	67	1	1	1	0						EUCAST	68	0	2	2	68	2
	100						1.1	24.5	71.3	1.1	1.1	1.1	0.0						4	72.3	0.0	2.1		72.3	2.1

Table A1.6 MIC distribution of all chicken *E. coli* (including those isolated from selective media) (2/2)

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																	Break point	Susceptibility			ECOFF		
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Florfenicol	64 100								2 3.1	8 12.5	13 20.3	5 7.8	5 7.8	7 10.9	24 37.5				N/A				16	33 51.6	31 48.4
Fosfomycin	64 100								39 60.9	13 20.3	3 4.7	1 1.6	1 1.6	1 1.6	1 1.6	5 7.8			EUCAST 64	58 90.6	0 0.0	6 9.4	4	55 85.9	9 14.1
Gentamicin	94 100						1 1.1	22 23.4	21 22.3	3 3.2	2 2.1	15 16.0	13 13.8	17 18.1					CLSI 16	49 52.1	15 16.0	30 31.9	2	47 50.0	47 50.0
Imipenem	64 100					33 51.6	31 48.4	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0					CLSI 4	64 100.0	0 0.0	0 0.0	0.5	64 100.0	0 0.0
Levofloxacin	64 100						20 31.3	8 12.5	4 6.3	4 6.3	9 14.1	19 29.7							CLSI 2	28 43.8	4 6.3	32 50.0	0.25	20 31.3	44 68.8
Meropenem	94 100				64 68.1	30 31.9	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0								CLSI 4	94 100.0	0 0.0	0 0.0	0.125	94 100.0	0 0.0
Nalidixic acid	94 100							0 0.0	1 1.1	15 16.0	17 18.1	4 4.3	4 4.3	3 3.2	50 53.2				CLSI 32	41 43.6	0 0.0	53 56.4	8	37 39.4	57 60.6
Nitrofurantoin	64 100												58 90.6	6 9.4	0 0.0	0 0.0			CLSI 128	64 100.0	0 0.0	0 0.0	64	64 100.0	0 0.0
Oxolinic acid	64 100	0 0.0	0 0.0	0 0.0	0 0.0	1 1.6	7 10.9	56 87.5											N/A				N/A		
Piperacillin/tazobactam	94 100								41 43.6	42 44.7	6 6.4	5 5.3	0 0.0	0 0					CLSI 128	94 100.0	0 0.0	0 0.0	8	94 100.0	0 0.0
Streptomycin	94 100									0 0.0	1 1.1	13 13.8	8 8.5	7 7.4	11 11.7	54 57.4			NARMS 32	22 23.4	0 0.0	72 76.6	16	22 23.4	72 76.6
Sulfisoxazole	94 100												20 21.3	3 3.2	0 0.0	2 2.1	2 2.1	67 71.3	CLSI 512	27 28.7	0 0.0	67 71.3	N/A		
Temocillin	64 100								1 1.6	10 15.6	31 48.4	20 31.3	2 3.125						EUCAST 32	0 0.0	64 100.0	0 0.0	16	64 100.0	0 0.0
Tetracycline	94 100										13 13.8	5 5.3	16 17.0	26 27.7	34 36.2				CLSI 16	13 13.8	5 5.3	76 80.9	8	18 19.1	76 80.9
Tigecycline	64 100						50 78.1	13 20.3	1 1.6	0 0.0	0 0.0	0 0.0							EUCAST 1	63 98.4	0 0.0	1 1.6	0.5	63 98.4	1 1.6
Trimethoprim/sulfamethoxazole	94 100					26 27.7	8 8.5	4 4.3	3 3.2	1 1.1	1 1.1	51 54.3							CLSI 4	42 44.7	0 0.0	52 55.3	0.25	34 36.2	60 63.8

Table A1.7 MIC distribution of pig *Salmonella*

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																Break point	Susceptibility			ECOFF		
		0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Amoxicillin/clavulanic acid	21 100							7 33.3	1 4.8	3 14.3	10 47.6	0 0.0	0 0.0					**CLSI 32**	21 100.0	0 0.0	0 0.0	**N/A**		
Ampicillin	21 100							7 33.3	0 0.0	1 4.8	1 4.8	0 0.0	0 0.0	12 57.1				**CLSI 32**	9 42.9	0 0.0	12 57.1	**8**	9 42.9	12 57.1
Azithromycin	21 100				0 0.0	0 0.0	0 0.0	0 0.0	1 4.8	10 47.6	5 23.8	3 14.3	2 9.5					**NARMS 32**	19 90.5	0 0.0	2 9.5	**16**	19 90.5	2 9.5
Cefoxitin	21 100						0 0.0	0 0.0	6 28.6	14 66.7	0 0.0	1 4.8	0 0.0					**CLSI 32**	20 95.2	1 4.8	0 0.0	**8**	20 95.2	1 4.8
Ceftazidime/avibactam	21 100							20 95.2	0 0.0	0 0.0	1 4.8	0 0.0						**CLSI 16**	21 100.0	0 0.0	0 0.0	**N/A**		
Ceftiofur	21 100				0 0.0	0 0.0	2 9.5	14 66.7	3 14.3	1 4.8	0 0.0	1 4.8						**NARMS 8**	19 90.5	1 4.8	1 4.8	**2**	19 90.5	2 9.5
Ceftolozane/tazobactam	21 100					0 0.0	16 76.2	4 19.0	1 4.8	0 0.0	0 0.0							**CLSI 8**	21 100.0	0 0.0	0 0.0	**N/A**		
Ceftriaxone	21 100					19 90.5	1 4.8	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	1 4.8			**CLSI 4**	20 95.2	0 0.0	1 4.8	**N/A**		
Chloramphenicol	21 100								0 0.0	1 4.8	9 42.9	2 9.5	1 4.8	8 38.1				**CLSI 32**	10 47.6	2 9.5	9 42.9	**16**	12 57.1	9 42.9
Ciprofloxacin	21 100	10 47.6	4 19.0	2 9.5	1 4.8	3 14.3	0 0.0	1 4.8	0 0.0	0 0.0								**CLSI 1**	16 76.2	4 19.0	1 4.8	**0.064**	16 76.2	5 23.8
Colistin	21 100					0 0.0	0 0.0	12 57.1	9 42.9	0 0.0	0 0.0							**EUCAST 4**	21 100.0	0 0.0	0 0.0	**N/A**		
Gentamicin	21 100					6 28.6	10 47.6	0 0.0	1 4.8	0 0.0	0 0.0	0 0.0	4 19.0					**CLSI 16**	17 81.0	0 0.0	4 19.0	**2**	17 81.0	4 19.0
Meropenem	21 100				21 100.0	0 0.0	0 0.0	0 0.0	0 0	0 0	0 0	0 0						**CLSI 4**	21 100.0	0 0.0	0 0.0	**N/A**		
Nalidixic acid	21 100						0 0.0	0 0.0	1 4.8	14 66.7	4 19.0	2 9.5	0 0.0					**NARMS 32**	21 100.0	0 0.0	0 0.0	**8**	19 90.5	2 9.5
Piperacillin/tazobactam	21 100							0 0.0	10 47.6	8 38.1	2 9.5	1 4.8	0 0.0					**CLSI 128**	21 100.0	0 0.0	0 0.0	**8**	20 95.2	1 4.8
Streptomycin	21 100								0 0.0	1 4.8	6 28.6	6 28.6	2 9.5	0 0.0	6 28.6			**NARMS 32**	13 61.9	0 0.0	8 38.1	**16**	13 61.9	8 38.1
Sulfisoxazole	21 100											2 9.5	5 23.8	4 19.0	0 0.0	0 0.0	10 47.6	**CLSI 512**	11 52.4	0 0.0	10 47.6	**N/A**		
Tetracycline	21 100									6 28.6	0 0.0	3 14.3	1 4.8	11 52.4				**CLSI 16**	6 28.6	0 0.0	15 71.4	**8**	6 28.6	15 71.4
Trimethoprim/sulfamethoxazole	21 100				11 52.4	3 14.3	0 0.0	0 0.0	0 0.0	0 0.0	7 33.3							**CLSI 4**	14 66.7	0 0.0	7 33.3	**N/A**		

Table A1.8 MIC distribution of chicken *Salmonella* (1/2)

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																	Break point	Susceptibility			ECOFF		
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Amikacin	17 100								16 94.1	1 5.9	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0				**CLSI 64**	17 100.0	0 0.0	0 0.0	**4**	17 100.0	0 0.0
Amoxicillin/clavulanic acid	24 100								19 79.2	0 0.0	0 0.0	0 0.0	5 20.8	0 0.0					**CLSI 32**	19 79.2	5 20.8	0 0.0	**N/A**		
Ampicillin	24 100								19 79.2	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	5 20.8				**CLSI 32**	19 79.2	0 0.0	5 20.8	**8**	19 79.2	5 20.8
Azithromycin	24 100						0 0.0	0 0.0	0 0.0	2 8.3	17 70.8	5 20.8	0 0.0	0 0.0					**NARMS 32**	24 100.0	0 0.0	0 0.0	**16**	24 100.0	0 0.0
Cefepime	17 100				15 88.2	1 5.9	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	1 5.9					**CLSI 16**	16 94.1	0 0.0	1 5.9	**N/A**		
Cefotaxime	17 100						16 94.1	0 0.0	0 0.0	0 0.0	0 0.0	1 5.9							**CLSI 4**	16 94.1	0 0.0	1 5.9	**0.5**	16 94.1	1 5.9
Cefoxitin	24 100							0 0.0	0 0.0	11 45.8	13 54.2	0 0.0	0 0.0	0 0.0					**CLSI 32**	24 100.0	0 0.0	0 0.0	**8**	24 100.0	0 0.0
Ceftazidime	17 100							16 94.1	0 0.0	0 0.0	0 0.0	0 0.0	1 5.9						**CLSI 16**	16 94.1	0 0.0	1 5.9	**2**	16 94.1	1 5.9
Ceftazidime/avibactam	7 100								7 100.0	0 0.0	0 0.0	0 0.0	0 0.0						**CLSI 16**	7 100.0	0 0.0	0 0.0	**N/A**		
Ceftiofur	24 100					0 0.0	0 0.0	10 41.7	13 54.2	0 0.0	0 0.0	0 0.0	1 4.2						**NARMS 8**	23 95.8	0 0.0	1 4.2	**2**	23 95.8	1 4.2
Ceftolozane/tazobactam	7 100						3 42.9	4 57.1	0 0.0	0 0.0	0 0.0	0 0.0							**CLSI 8**	7 100.0	0 0.0	0 0.0	**N/A**		
Ceftriaxone	24 100						23 95.8	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	1 4.2			**CLSI 4**	23 95.8	0 0.0	1 4.2	**N/A**		
Chloramphenicol	24 100									0 0.0	4 16.7	14 58.3	2 8.3	0 0.0	4 16.7				**CLSI 32**	18 75.0	2 8.3	4 16.7	**16**	20 83.3	4 16.7
Ciprofloxacin	24 100		18 75.0	1 4.2	0 0.0	0 0.0	0 0.0	4 16.7	0 0.0	0 0.0	0 0.0	1 4.2							**CLSI 1**	19 79.2	4 16.7	1 4.2	**0.064**	19 79.2	5 20.8
Colistin	24 100								19 79.2	5 20.8	0 0.0	0 0.0	0 0.0						**EUCAST 4**	24 100.0	0 0.0	0 0.0	**N/A**		

Table A1.8 MIC distribution of chicken *Salmonella* (2/2)

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																	Break point	Susceptibility			ECOFF		
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Florfenicol	17 100								1 5.9	1 5.9	12 70.6	0 0.0	0 0.0	1 5.9	2 11.8				CLSI V 16	14 82.4	0 0.0	3 17.6	16	14 82.4	3 17.6
Fosfomycin	17 100								14 82.4	1 5.9	2 11.8	0 0.0	0 0.0	0 0.0	0 0.0				EUCAST 64	17 100.0	0 0.0	0 0.0	N/A		
Gentamicin	24 100						10 41.7	12 50.0	0 0.0	0 0.0	0 0.0	0 0.0	1 4.2	1 4.2					CLSI 16	22 91.7	0 0.0	2 8.3	2	22 91.7	2 8.3
Imipenem	17 100					0 0.0	15 88.2	2 11.8	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0						CLSI 4	17 100.0	0 0.0	0 0.0	1	17 100.0	0 0.0
Levofloxacin	17 100						14 82.4	2 11.8	1 5.9	0 0.0	0 0.0								CLSI 2	0 0.0	17 100.0	0 0.0	0.25	14 82.4	3 17.6
Meropenem	24 100				17 70.8	7 29.2	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0								CLSI 4	24 100.0	0 0.0	0 0.0	N/A		
Nalidixic acid	24 100							0 0.0	0 0.0	2 8.3	17 70.8	1 4.2	1 4.2	0 0.0	3 12.5				CLSI 32	21 87.5	0 0.0	3 12.5	8	20 83.3	4 16.7
Nitrofurantoin	17 100												6 35.3	11 64.7	0 0.0	0 0.0			CLSI 128	17 100.0	0 0.0	0 0.0	N/A		
Oxolinic acid	17 100	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	5 29.4	12 70.6											N/A				N/A		
Piperacillin/tazobactam	24 100								0 0.0	19 79.2	2 8.3	1 4.2	0 0.0	2 8.3					CLSI 128	22 91.7	2 8.3	0 0.0	8	22 91.7	2 8.3
Streptomycin	24 100									4 16.7	2 8.3	8 33.3	4 16.7	3 12.5	0 0.0	3 12.5			NARMS 32	18 75.0	0 0.0	6 25.0	16	18 75.0	6 25.0
Sulfisoxazole	24 100												2 8.3	14 58.3	2 8.3	0 0.0	4 16.7	2 8.3	CLSI 512	22 91.7	0 0.0	2 8.3	N/A		
Temocillin	17 100								0 0.0	0 0.0	5 29.4	9 52.9	3 17.6						N/A				N/A		
Tetracycline	24 100										17 70.8	0 0.0	0 0.0	0 0.0	7 29.2				CLSI 16	17 70.8	0 0.0	7 29.2	8	17 70.8	7 29.2
Tigecycline	17 100						12 70.6	5 29.4	0 0.0	0 0.0	0 0.0	0 0.0							FDA 8	17 100.0	0 0.0	0 0.0	N/A		
Trimethoprim/sulfamethoxazole	24 100					18 75.0	2 8.3	0 0.0	0 0.0	0 0.0	0 0.0	4 16.7							CLSI 4	20 83.3	0 0.0	4 16.7	N/A		

Table A1.9 MIC distribution of pig *Enterococcus faecalis*

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																			Break point	Susceptibility			ECOFF		
		0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096		S	I	R		W	NW
Ampicillin	4 100					1 25.0	2 50.0	1 25.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0								**CLSI 16**	4 100.0	0 0.0	0 0.0	**4**	4 100.0	0 0.0
Avilamycin	4 100									4 100.0	0 0.0	0 0.0	0 0.0								**N/A**				**8**	4 100.0	0 0.0
Chloramphenicol	4 100								0 0.0	1 25.0	1 25.0	1 25.0	1 25.0	0 0.0							**CLSI 32**	2 50.0	1 25.0	1 25.0	**32**	4 100.0	0 0.0
Ciprofloxacin	4 100				0 0.0	0 0.0	2 50.0	2 50.0	0 0.0	0 0.0	0 0.0	0 0.0									**CLSI 4**	4 100.0	0 0.0	0 0.0	**4**	4 100.0	0 0.0
Daptomycin	4 100					0 0.0	0 0.0	2 50.0	1 25.0	1 25.0	0 0.0	0 0.0									**CLSI 8**	3 75.0	1 25.0	0 0.0	**4**	4 100.0	0 0.0
Erythromycin	4 100					0 0.0	0 0.0	2 50.0	0 0.0	1 25.0	0 0.0	1 25.0									**CLSI 8**	0 0.0	3 75.0	1 25.0	**4**	3 75.0	1 25.0
Gentamicin	4 100											3 75.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	1 25.0		**EUCAST 256**	3 75.0	0 0.0	1 25.0	**64**	3 75.0	1 25.0
Linezolid	4 100						0 0.0	0 0.0	3 75.0	1 25.0	0 0.0										**CLSI 8**	3 75.0	1 25.0	0 0.0	**4**	4 100.0	0 0.0
Nitrofurantoin	4 100								0 0.0	0 0.0	1 25.0	2 50.0	0 0.0	1 25.0	0 0.0	0 0.0	0 0.0				**CLSI 128**	3 75.0	1 25.0	0 0.0	**32**	3 75.0	1 25.0
Quinupristin/dalfopristin	4 100						0 0.0	0 0.0	0 0.0	1 25.0	1 25.0	2 50.0	0 0.0								**N/A**				**N/A**		
Streptomycin	4 100													3 75.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	1 25.0	**EUCAST 1024**	3 75.0	0 0.0	1 25.0	**512**	3 75.0	1 25.0
Tetracycline	4 100							1 25.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	3 75.0							**CLSI 16**	1 25.0	0 0.0	3 75.0	**4**	1 25.0	3 75.0
Tigecycline	4 100	0 0.0	0 0.0	0 0.0	3 75.0	1 25.0	0 0.0														**EUCAST 0.5**	4 100.0	0 0.0	0 0.0	**0.25**	4 100.0	0 0.0
Vancomycin	4 100					0 0.0	1 25.0	1 25.0	0 0.0	2 50.0	0 0.0	0 0.0	0 0.0								**CLSI 32**	4 100.0	0 0.0	0 0.0	**4**	4 100.0	0 0.0

Table A1.10 MIC distribution of chicken *Enterococcus faecalis*

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																			Break point	Susceptibility			ECOFF		
		0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096		S	I	R		W	NW
Ampicillin	23 100					0 0.0	2 8.7	20 87.0	1 4.3	0 0.0	0 0.0	0 0.0	0 0.0								**CLSI 16**	23 100.0	0 0.0	0 0.0	**4**	23 100.0	0 0.0
Avilamycin	23 100									23 100.0	0 0.0	0 0.0	0 0.0								**N/A**				**8**	23 100.0	0 0.0
Chloramphenicol	23 100								0 0.0	2 8.7	15 65.2	0 0	3 13.0	1 4.3	2 8.7						**CLSI 32**	17 73.9	0 0.0	6 26.1	**32**	20 87.0	3 13.0
Ciprofloxacin	23 100				0 0.0	0 0.0	0 0.0	8 34.8	0 0.0	0 0.0	0 0.0	7 30.4	8 34.8								**CLSI 4**	8 34.8	0 0.0	15 65.2	**4**	8 34.8	15 65.2
Daptomycin	23 100					0 0.0	1 4.3	8 34.8	13 56.5	1 4.3	0 0.0	0 0.0									**CLSI 8**	22 95.7	1 4.3	0 0.0	**4**	23 100.0	0 0.0
Erythromycin	23 100					0 0.0	0 0.0	0 0.0	1 4.3	0 0.0	0 0.0	22 95.7									**CLSI 8**	0 0.0	1 4.3	22 95.7	**4**	1 4.3	22 95.7
Gentamicin	23 100											10 43.5	0 0.0	0 0.0	0 0.0	2 8.7	4 17.4	3 13.0	4 17.4		**EUCAST 256**	10 43.5	0 0.0	13 56.5	**64**	10 43.5	13 56.5
Linezolid	23 100						2 8.7	7 30.4	12 52.2	2 8.7	0 0.0										**CLSI 8**	21 91.3	2 8.7	0 0.0	**4**	23 100.0	0 0.0
Nitrofurantoin	23 100								2 8.7	0 0.0	12 52.2	8 34.8	1 4.3	0 0.0	0 0.0	0 0.0	0 0.0				**CLSI 128**	23 100.0	0 0.0	0 0.0	**32**	23 100.0	0 0.0
Quinupristin/dalfopristin	23 100						0 0.0	0 0.0	0 0.0	1 4.3	2 8.7	19 82.6	1 4.3								**N/A**				**N/A**		
Streptomycin	23 100													7 30.4	0 0.0	0 0.0	0 0.0	0 0.0	12 52.2	4 17.4	**EUCAST 1024**	7 30.4	0 0.0	16 69.6	**512**	7 30.4	16 69.6
Tetracycline	23 100							2 8.7	0 0.0	0 0.0	0 0.0	0 0.0	1 4.3	20 87.0							**CLSI 16**	2 8.7	0 0.0	21 91.3	**4**	2 8.7	21 91.3
Tigecycline	23 100	0 0.0	0 0.0	0 0.0	2 8.7	20 87.0	1 4.3														**EUCAST 0.5**	22 95.7	0 0.0	1 4.3	**0.25**	22 95.7	1 4.3
Vancomycin	23 100					0 0.0	0 0.0	20 87.0	2 8.7	1 4.3	0 0.0	0 0.0	0 0.0								**CLSI 32**	23 100.0	0 0.0	0 0.0	**4**	23 100.0	0 0.0

Table A1.11 MIC distribution of pig *Enterococcus faecium*

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																			Break point	Susceptibility			ECOFF		
		0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096		S	I	R		W	NW
Ampicillin	16 100					1 6.3	0 0.0	5 31.3	9 56.3	0 0.0	1 6.3	0 0.0	0 0.0								**CLSI 16**	16 100.0	0 0.0	0 0.0	**4**	15 93.8	1 6.3
Avilamycin	16 100									16 100.0	0 0.0	0 0.0	0 0.0								**N/A**				**16**	16 100.0	0 0.0
Chloramphenicol	16 100								0 0.0	3 18.8	9 56.3	3 18.8	0 0.0	1 6.3							**CLSI 32**	12 75.0	3 18.8	1 6.3	**32**	15 93.8	1 6.3
Ciprofloxacin	16 100				0 0.0	0 0.0	4 25.0	10 62.5	2 12.5	0 0.0	0 0.0	0 0.0									**CLSI 4**	14 87.5	2 12.5	0 0.0	**8**	16 100.0	0 0.0
Daptomycin	16 100					0 0.0	0 0.0	1 6.3	3 18.8	11 68.8	1 6.3	0 0.0									**CLSI 8**	15 93.8	0 0.0	1 6.3	**8**	16 100.0	0 0.0
Erythromycin	16 100					5 31.3	0 0.0	0 0.0	5 31.3	3 18.8	1 6.3	2 12.5									**CLSI 8**	5 31.3	8 50.0	3 18.8	**4**	13 81.3	3 18.8
Gentamicin	16 100											16 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0			**EUCAST 256**	16 100.0	0 0.0	0 0.0	**32**	16 100.0	0 0.0
Linezolid	16 100						1 6.3	0 0.0	13 81.3	2 12.5	0 0.0										**CLSI 8**	14 87.5	2 12.5	0 0.0	**4**	16 100.0	0 0.0
Nitrofurantoin	16 100								1 6.3	0 0.0	0 0.0	0 0.0	3 18.8	10 62.5	2 12.5	0 0.0	0 0.0				**CLSI 128**	4 25.0	10 62.5	2 12.5	**256**	16 100.0	0 0.0
Quinupristin/dalfopristin	16 100						0 0.0	0 0.0	0 0.0	14 87.5	2 12.5	0 0.0	0 0.0								**EUCAST 8**	0 0.0	14 87.5	2 12.5	**N/A**		
Streptomycin	16 100													13 81.3	0 0.0	0 0.0	0 0.0	2 12.5	1 6.3	0 0.0	**EUCAST 1024**	13 81.3	0 0.0	3 18.8	**128**	13 81.3	3 18.8
Tetracycline	16 100							8 50.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	8 50.0							**CLSI 16**	8 50.0	0 0.0	8 50.0	**4**	8 50.0	8 50.0
Tigecycline	16 100	0 0.0	1 6.3	2 12.5	9 56.3	4 25.0	0 0.0														**EUCAST 0.5**	16 100.0	0 0.0	0 0.0	**0.25**	16 100.0	0 0.0
Vancomycin	16 100					0 0.0	9 56.3	7 43.8	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0								**CLSI 32**	16 100.0	0 0.0	0 0.0	**4**	16 100.0	0 0.0

Table A1.12 MIC distribution of pig *Campylobacter coli*

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:														Break point	Susceptibility			ECOFF		
		0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128		S	I	R		W	NW
Azithromycin	8	0	0	2	3	0	0	0	0	0	0	0	0	0	3	N/A				0.5	5	3
	100	0.0	0.0	25.0	37.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	37.5						62.5	37.5
Ciprofloxacin	8	0	0	0	0	0	0	0	0	0	4	3	1	0		CLSI 4	0	0	8	0.5	0	8
	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0	37.5	12.5	0.0			0.0	0.0	100.0		0.0	100.0
Clindamycin	8		0	0	0	1	2	0	1	2	2	0				N/A				1	3	5
	100		0.0	0.0	0.0	12.5	25.0	0.0	12.5	25.0	25.0	0.0									37.5	62.5
Erythromycin	8		0	0	0	0	2	2	1	0	0	0	0	0	3	CLSI 32	5	0	3	8	5	3
	100		0.0	0.0	0.0	0.0	25.0	25.0	12.5	0.0	0.0	0.0	0.0	0.0	37.5		62.5	0.0	37.5		62.5	37.5
Florfenicol	8		0	0	0	0	1	0	1	2	4	0	0	0		N/A				4	4	4
	100		0.0	0.0	0.0	0.0	12.5	0.0	12.5	25.0	50.0	0.0	0.0	0.0							50.0	50.0
Gentamicin	8				0	0	0	5	0	0	0	0	0	3		N/A				1	5	3
	100				0.0	0.0	0.0	62.5	0.0	0.0	0.0	0.0	0.0	37.5							62.5	37.5
Nalidixic Acid	8									0	0	0	0	2	6	N/A				16	0	8
	100									0.0	0.0	0.0	0.0	25.0	75.0						0.0	100.0
Tetracycline	8			0	0	0	0	0	0	0	0	0	1	2	5	CLSI 16	0	0	8	2	0	8
	100			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.5	25.0	62.5		0.0	0.0	100.0		0.0	100.0

Table A1.13 MIC distribution of fish *Vibrio* spp. (1/2)

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																	Break point	Susceptibility			ECOFF		
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Ampicillin	22 100								1 4.5	0 0.0	1 4.5	0 0.0	3 13.6	17 77.3					CLSI 32	2 9.1	3 13.6	17 77.3	N/A		
Amoxicillin/clavulanic acid	22 100								0 0.0	3 13.6	15 68.2	4 18.2	0 0.0	0 0.0					CLSI 32	22 100.0	0 0.0	0 0.0	N/A		
Piperacillin/ tazobactam	22 100								21 95.5	0 0.0	1 4.5	0 0.0	0 0.0	0 0.0					CLSI 128	22 100.0	0 0.0	0 0.0	N/A		
Meropenem	22 100				21 95.5	0 0.0	1 4.5	0 0.0	0 0.0	0 0.0	0 0.0								CLSI 4	22 100.0	0 0.0	0 0.0	N/A		
Cefoxitin	22 100							0 0.0	0 0.0	0 0.0	0 0.0	5 22.7	14 63.6	3 13.6					CLSI 32	5 22.7	14 63.6	3 13.6	N/A		
Ceftiofur	22 100					1 4.5	1 4.5	4 18.2	16 72.7	0 0.0	0 0.0	0 0.0							N/A				N/A		
Ceftriaxone	22 100						22 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0				N/A				N/A		
Ceftazidime	22 100							22 100.0	0 0.0	0 0.0	0 0.0	0 0.0							CLSI 16	22 100.0	0 0.0	0 0.0	N/A		
Colistin	21 100								3 14.3	0 0.0	0 0.0	2 9.5	16 76.2						N/A				N/A		
Gentamicin	22 100						0 0.0	4 18.2	6 27.3	9 40.9	3 13.6	0 0.0	0 0.0						CLSI 16	22 100.0	0 0.0	0 0.0	N/A		
Streptomycin	22 100									4 18.2	1 4.5	5 22.7	6 27.3	5 22.7	1 4.5				N/A				N/A		
Sulfisoxazole	22 100												11 50.0	4 18.2	4 18.2	1 4.5	2 9.1		N/A				N/A		
Trimethoprim/sulfamethoxazole	22 100					20 90.9	1 4.5	0 0.0	0 0.0	1 4.5	0 0.0								CLSI 4	22 100.0	0 0.0	0 0.0	N/A		
Ciprofloxacin	22 100		4 18.2	1 4.5	2 9.1	5 22.7	9 40.9	1 4.5	0 0.0	0 0.0	0 0.0								CLSI 4	22 100.0	0 0.0	0 0.0	N/A		
Nalidixic Acid	22 100							11 50.0	9 40.9	2 9.1	0 0.0	0 0.0	0 0.0	0 0.0					N/A				N/A		

Table A1.13 MIC distribution of fish *Vibrio* spp. (2/2)

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																	Break point	Susceptibility			ECOFF		
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Azithromycin	22 100						7 31.8	4 18.2	10 45.5	1 4.5	0 0.0	0 0.0	0 0.0	0 0.0					N/A				N/A		
Tetracycline	22 100										22 100.0	0 0.0	0 0.0	0 0.0					CLSI 16	22 100.0	0 0.0	0 0.0	N/A		
Chloramphenicol	22 100									22 100.0	0 0.0	0 0.0	0 0.0	0 0.0					N/A				N/A		
Amikacin	22 100								0 0.0	4 18.2	8 36.4	7 31.8	3 13.6	0 0.0	0 0.0				CLSI 64	22 100.0	0 0.0	0 0.0	N/A		
Cefepime	22 100				1 4.5	0 0.0	3 13.6	12 54.5	6 27.3	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0					CLSI 16	22 100.0	0 0.0	0 0.0	N/A		
Cefotaxime	22 100						22 100.0	0 0.0	0 0.0	0 0.0	0 0.0								CLSI 4	22 100.0	0 0.0	0 0.0	N/A		
Florfenicol	22 100								22 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0					N/A				N/A		
Fosfomycin	22 100								7 31.8	4 18.2	9 40.9	1 4.5	0 0.0	1 4.5	0 0.0				N/A				N/A		
Imipenem	22 100					11 50.0	10 45.5	0 0.0	1 4.5	0 0.0	0 0.0	0 0.0	0 0.0						CLSI 4	22 100.0	0 0.0	0 0.0	N/A		
Levofloxacin	22 100						19 86.4	3 13.6	0 0.0	0 0.0	0 0.0								CLSI 8	22 100.0	0 0.0	0 0.0	N/A		
Nitrofurantoin	22 100												22 100.0	0 0.0	0 0.0	0 0.0			N/A				N/A		
Oxolinic avid	22 100	0 0.0	0 0.0	2 9.1	4 18.2	5 22.7	11 50.0												N/A				N/A		
Temocillin	22 100							2 9.1	16 72.7	4 18.2	0 0.0	0 0.0	0 0.0						N/A				N/A		
Tigecycline	22 100						22 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0							N/A				N/A		

Table A1.14 MIC distribution of fish *Photobacterium* spp. (1/2)

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																	Break point	Susceptibility			ECOFF		
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Ampicillin	25 100								14 56.0	6 24.0	4 16.0	0 0.0	0 0.0	1 4.0					N/A				N/A		
Amoxicillin/clavulanic acid	25 100								24 96.0	0 0.0	0 0.0	0 0.0	1 4.0	0 0.0					N/A				N/A		
Piperacillin/ tazobactam	25 100								24 96.0	1 4.0	0 0.0	0 0.0	0 0.0	0 0.0					N/A				N/A		
Meropenem	25 100				25 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0								N/A				N/A		
Cefoxitin	25 100							2 8.0	10 40.0	12 48.0	0 0.0	1 4.0	0 0.0	0 0.0					N/A				N/A		
Ceftiofur	25 100					24 96.0	0 0.0	0 0.0	0 0.0	1 4.0	0 0.0	0 0.0							N/A				N/A		
Ceftriaxone	25 100						25 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0				N/A				N/A		
Ceftazidime	25 100							25 100.0	0 0.0	0 0.0	0 0.0	0 0.0							N/A				N/A		
Colistin	25 100								21 84.0	1 4.0	1 4.0	0 0.0	2 8.0						N/A				N/A		
Gentamicin	25 100						4 16.0	15 60.0	6 24.0	0 0.0	0 0.0	0 0.0	0 0.0						N/A				N/A		
Streptomycin	25 100									3 12.0	17 68.0	4 16.0	1 4.0	0 0.0	0 0.0				N/A				N/A		
Sulfisoxazole	25 100												0 0.0	1 4.0	0 0.0	15 60.0	9 36.0		N/A				N/A		
Trimethoprim/sulfamethoxazole	25 100					24 96.0	0 0.0	1 4.0	0 0.0	0 0.0	0 0.0								N/A				N/A		
Ciprofloxacin	25 100		25 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0								N/A				N/A		
Nalidixic Acid	25 100							25 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0					N/A				N/A		

Table A1.14 MIC distribution of fish *Photobacterium* spp. (2/2)

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																	Break point	Susceptibility			ECOFF		
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Azithromycin	25 100						23 92.0	1 4.0	0 0.0	0 0.0	1 4.0	0 0.0	0 0.0	0 0.0					N/A				N/A		
Tetracycline	25 100										25 100.0	0 0.0	0 0.0	0 0.0					N/A				N/A		
Chloramphenicol	25 100									22 88.0	3 12.0	0 0.0	0 0.0	0 0.0					N/A				N/A		
Amikacin	25 100								2 8.0	13 52.0	10 40.0	0 0.0	0 0.0	0 0.0	0 0.0				N/A				N/A		
Cefepime	25 100				9 36.0	13 52.0	3 12.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0					N/A				N/A		
Cefotaxime	25 100						25 100.0	0 0.0	0 0.0	0 0.0	0 0.0								N/A				N/A		
Florfenicol	25 100								25 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0					N/A				N/A		
Fosfomycin	25 100								23 92.0	0 0.0	1 4.0	0 0.0	0 0.0	1 4.0	0 0.0				N/A				N/A		
Imipenem	25 100					21 84.0	4 16.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0						N/A				N/A		
Levofloxacin	25 100						25 100.0	0 0.0	0 0.0	0 0.0	0 0.0								N/A				N/A		
Nitrofurantoin	25 100												24 96.0	1 4.0	0 0.0	0 0.0			N/A				N/A		
Oxolinic avid	25 100	0 0.0	0 0.0	10 40.0	15 60.0	0 0.0	0 0.0												N/A				N/A		
Temocillin	25 100							22 88.0	2 8.0	1 4.0	0 0.0	0 0.0	0 0.0						N/A				N/A		
Tigecycline	25 100						24 96.0	1 4.0	0 0.0	0 0.0	0 0.0	0 0.0							N/A				N/A		

Table A1.15 MIC distribution of fish *Aeromonas* spp. (1/2)

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																	Break point	Susceptibility			ECOFF		
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Ampicillin	5 100								1 20.0	1 20.0	0 0.0	0 0.0	0 0.0	3 60.0					N/A				N/A		
Amoxicillin/clavulanic acid	5 100								2 40.0	0 0.0	0 0.0	1 20.0	2 40.0	0 0.0					N/A				N/A		
Piperacillin/ tazobactam	5 100								2 40.0	1 20.0	2 40.0	0 0.0	0 0.0	0 0.0					CLSI 128	5 100.0	0 0.0	0 0.0	N/A		
Meropenem	5 100				3 60.0	2 40.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0								CLSI 4	5 100.0	0 0.0	0 0.0	N/A		
Cefoxitin	5 100							3 60.0	0 0.0	2 40.0	0 0.0	0 0.0	0 0.0	0 0.0					CLSI 32	5.0 100.0	0 0.0	0 0.0	N/A		
Ceftiofur	5 100					1 20.0	1 20.0	1 20.0	2 40.0	0 0.0	0 0.0	0 0.0							N/A				N/A		
Ceftriaxone	5 100						5 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0				CLSI 4	5 100.0	0 0.0	0 0.0	N/A		
Ceftazidime	5 100							5 100.0	0 0.0	0 0.0	0 0.0	0 0.0							CLSI 16	5.0 100.0	0 0.0	0 0.0	N/A		
Colistin	5 100								5 100.0	0 0.0	0 0.0	0 0.0	0 0.0						N/A				N/A		
Gentamicin	5 100						0 0.0	3 60.0	1 20.0	1 20.0	0 0.0	0 0.0	0 0.0						CLSI 16	5 100.0	0 0.0	0 0.0	N/A		
Streptomycin	5 100									1 20.0	1 20.0	0 0.0	2 40.0	1 20.0	0 0.0				N/A				N/A		
Sulfisoxazole	5 100												2 40.0	1 20.0	1 20.0	1 20.0	0.0 0.0		N/A				N/A		
Trimethoprim/sulfamethoxazole	5 100					5 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0								CLSI 4	5 100.0		0 0.0	N/A		
Ciprofloxacin	5 100		5 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0								CLSI 4	5 100.0	0 0.0	0 0.0	N/A		
Nalidixic Acid	5 100							5 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0					N/A				N/A		

Table A1.15 MIC distribution of fish *Aeromonas* spp. (2/2)

Antimicrobial	Total n %	Number and precentage of isolates with MICs (mg/L) at:																	Break point	Susceptibility			ECOFF		
		0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512		S	I	R		W	NW
Azithromycin	5 100						1 20.0	0 0.0	3 60.0	1 20.0	0 0.0	0 0.0	0 0.0	0 0.0					N/A				N/A		
Tetracycline	5 100										5 100.0	0 0.0	0 0.0	0 0.0					CLSI 16	5 100.0	0 0.0	0 0.0	N/A		
Chloramphenicol	5 100									5 100.0	0 0.0	0 0.0	0 0.0	0 0.0					CLSI 32	5 100.0	0 0.0	0 0.0	N/A		
Amikacin	5 100								1 20.0	2 40.0	1 20.0	1 20.0	0 0.0	0 0.0	0 0.0				CLSI 64	5 100.0	0 0.0	0 0.0	N/A		
Cefepime	5 100				4 80.0	1 20.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0					CLSI 16	5 100.0	0 0.0	0 0.0	N/A		
Cefotaxime	5 100						5 100.0	0 0.0	0 0.0	0 0.0	0 0.0								CLSI 4	5 100.0	0 0.0	0 0.0	N/A		
Florfenicol	5 100								5 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0					N/A				N/A		
Fosfomycin	5 100								1 20.0	3 60.0	0 0.0	0 0.0	0 0.0	0 0.0	1 20.0				N/A				N/A		
Imipenem	5 100					2 40.0	1 20.0	1 20.0	1 20.0	0 0.0	0 0.0	0 0.0	0 0.0						CLSI 4	5 100.0	0 0.0	0 0.0	N/A		
Levofloxacin	5 100						5 100.0	0 0.0	0 0.0	0 0.0	0 0.0								CLSI 8	5 100.0	0 0.0	0 0.0	N/A		
Nitrofurantoin	5 100												5 100.0	0 0.0	0 0.0	0 0.0			N/A				N/A		
Oxolinic avid	5 100	0 0.0	2 40.0	2 40.0	1 20.0	0 0.0	0 0.0												N/A				N/A		
Temocillin	5 100							2 40.0	1 20.0	2 40.0	0 0.0	0 0.0	0 0.0						N/A				N/A		
Tigecycline	5 100						5 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0							N/A				N/A		

Annex 2 Methods

A2.1 Antimicrobial usage – pigs and chickens

A2.1.1 AMU data collection

Farmers reported on AMU voluntarily and agreed/were expected to provide information on a monthly basis. A simple AMU report form was devised for farmers to facilitate easy reporting. The key AMU data collected were the quantity of each AM product used and the concentration of the active ingredient. The AMU report forms were typically submitted in person during farm visits, or via fax/instant messages. Farmers who missed reporting were reminded by phone calls and/or instant messages.

A2.1.2 AMU data collation and calculation

The submitted AMU data were collated and recorded in an excel spreadsheet in standardized format to facilitate subsequent AMU calculations in different metrics in a tailor-made Access database. Each farm was assigned a special farm code to maintain anonymity. The relevant data recorded for AMU calculations include the active ingredient, antimicrobial class, reported usage in ml (solution) or kg (solid), and concentration in mg/ml (solution) or mg/kg (solid).

The resulting usage metrics include quantity of active AM ingredient in kg, mg/kg TAB (target animal biomass), mg/kg PCU (population correction unit), and DDDvet/1000 animal-days at risk. The calculation of the latter three metrics requires the slaughter number and slaughter weight. The slaughter number of pigs were derived from statistics from the Sheung Shui Slaughterhouse from the Food and Environmental Hygiene Department (FEHD). The slaughter number of chickens was derived from statistics from the Cheung Sha Wan Temporary Wholesale Poultry Market of the AFCD.

For the calculation of mg/kg TAB, the slaughter weight of the animals were estimated based on local knowledge, at 130kg for finisher pigs, 240kg for sows, 1.75kg for broiler chickens, and 3kg for breeder chickens. For the calculation of mg/kg PCU, the ESVAC (European Surveillance of Veterinary Antimicrobial Consumption) estimated weights at time of treatment were used (i.e. 65kg for finisher pigs, 240kg for sows, and 1kg for broiler chickens).

For the calculation of DDDvet/1000 animal-days at risk, the DDDvet (defined daily doses for animals) values for each animal-substance-route combination were applied in the following orders, when available: 1) ESVAC DDDvet values, 2) product dose as

recommended by the manufacturer, and 3) appropriate dose with reference to literature. The PCU weights were used in the calculation of DDDvet/1000 animal-days at risk. The days at risk, estimated based on local knowledge, were set at 200 days for finisher pigs, 365 days for sows, and 90 days for broiler chickens.

Table A2.1 Strengths and weaknesses of AMU metrics and reasons for adoption

Metric	Pros	Cons	Comment
1. Overall quantities (kg) total and by class by production type	Relatively easy to measure if farmers continue to provide reports	No denominator When comparing quantities by class information on the manner of administration is not included in the metric	Production of food animals in Hong Kong has been relatively stable and if this trend continues the data can still be interpreted even without a denominator
2. Overall quantities (kg) total and by class divided by TAB (kg)	Provides better information if there is a marked change in levels of production from year to year Essential for comparing use between farms of different sizes	Gives a lower figure than PCU Does not provide information on when treatment was given	The most important metric given it also includes a denominator that represents the total biomass produced per annum
3. Overall quantities (kg) total and by class divided by PCU (kg)	Provides capacity to compare with European values Might to some extent better represent the weight at which treatment occurs	PCU does not reflect local production systems Does not fully reflect the weight at which treatment occurs	Only recommending use because it is widely used elsewhere. It may provide some crude comparisons with levels used in other countries but these are not strictly valid given the differences in production systems in Hong Kong
4. DDDvet per 1000 animal-days at risk	Takes into account that the dose given for some AMs is greater than others Provides some comparability with metrics used in humans Used in Europe	Is not an exact match for DDD in humans	Not needed if data on individual classes of AMs is separated – already well recognised that in-feed dose often higher than parenteral dose and that dose of active ingredient for some AM is higher than others Included because it is calculated elsewhere – same caveats apply for denominator as for (3) above. DDDs derived from Europe and may not be directly applicable to Hong Kong. Some DDD not available for AMs not used in Europe in food animals

A2.2 AMU audit testing – pigs and chickens

A2.2.1 Feed sample collection

Grower or finisher feed not known to contain AMs were targeted for collection. Approximately 500g of ready-to-feed compound feed samples were collected into a new Ziploc bag, either by pouring directly into the bag from the feed mixer, or by hand in clean disposable gloves from feed troughs. Occasionally farmers may use their own farm tools to assist with the feed collection. The feed samples were then stored and refrigerated at 4oC for up to 3 months from collection. If the samples were likely to be stored for more than 3 months prior to dispatch for testing, then the sample would be frozen at -18oC instead. When ready to be shipped, the samples were transferred to a new, lidded plastic container for shipping at ambient temperature to Wageningen University and Research (WUR) in the Netherlands.

A2.2.2 Feed testing for AM residues

Compound feed samples were tested by the Wageningen University and Research (WUR) in Netherlands, a Dutch National Reference Laboratory (NRL) for monitoring residues and contaminants in animal feed. WUR used a microbiological screening test, followed by selected confirmatory liquid chromatography–mass spectrometry (LC-MS) tests on any samples producing inhibition of bacterial growth. The levels of heavy metals such as zinc and copper in feed were also determined using atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS).

A2.2.3 Faecal waste sample collection

Faecal waste samples collected from pigs farms were typically from solid waste separator and occasionally from waste bins, whereas in chicken farms the samples were collected either from waste bins or under the cages. The specific collection site varied depending on accessibility and availability of waste bins, which may have been emptied. Approximately 300g of faecal waste was collected using a new, disposable plastic spoon into a clean, lidded plastic bottle. Collected samples were stored in a chilled bin with ice and then transported to local facility for storage in freezer at -20oC until submission, also in chilled bin with ice, as a batch to the laboratory on a weekly basis.

A2.2.4 Faecal waste testing for AM residues

The testing of faecal waste for the presence of AM residues was conducted as a trial as the methods were being developed. Samples were prepared by freeze-drying, chemical extraction and solid-phase extraction. Liquid chromatography-tandem mass

spectrometry (LC-MS/MS) was then used for the detection and quantification of AM residues.

A2.3 Antimicrobial resistance – pigs and chickens

A2.3.1 AMR sample collection

Samples for AMR testing were collected from active pig farms and active chicken farms. A minimum of two sample sets were collected in each farm visit.

For pig farms, fresh faeces on the ground were collected with a new, disposable plastic spoon into a new plastic bottle from different sheds/barns of market weight animals. Each sample contained approximately 10g of faeces from a single animal. The sheds/barns were randomly chosen based on accessibility and availability of fresh faeces. Collected samples were stored in a chilled bin with ice and then transported to the laboratory.

For chicken farms, cloacal swabs and environmental drag swabs were collected randomly from market weight animals and the relevant house, respectively. Cloacal samples were preferentially collected from different houses, or at least from distant cages if in the same house. Environmental drag swabs were collected under/near where the cloacal samples were taken. Collected samples were stored in a chilled bin with ice and then transported to the laboratory.

A2.3.2 Bacterial culture

The culture methods used to screen for targeted organisms strictly follow a designated protocol designed by Veterinary Laboratory Division of AFCD (accredited laboratory by National Association of Testing Authorities, Australia) based on relevant international standards. All poultry and pig samples are screened for indicator *E. coli*, *Salmonella* spp., *Campylobacter* spp., *Enterococcus faecium* and *E. faecalis* (in addition, *E. avium* for poultry samples). Selective media were used for isolation of suspected ESBL/AmpC-producing Enterobacteriaceae, and Carbapenemase-producing gram negative organism.

A2.3.3 Microbiological methods

A2.3.3.1 *Escherichia coli* (indicator)

Indicator *E. coli* from broilers and pigs was isolated by adding 10g samples in 2% buffered peptone water (BPW) (Oxoid) and incubated at 37°C for 18-22hrs. One loop (10uL) of the overnight culture was then directly plated onto a MacConkey agar plate

and further incubated at 37°C for 18-22 hours. Only one indicator *E. coli* isolate per faecal sample of pig and cloacal swab sample from poultry was selected.

For specific isolation of suspected ESBL/AmpC and carbapenemase-producing *E. coli* from faecal/swab samples, the present EURL-AR laboratory protocols describing the selective enrichment procedures was applied. Commercial selective agar (Brilliance ESBL agar (ThermoFisher Scientific) and CHROMID CARBA SMART agar (BioMerieux)) were used. Only one potential ESBL/AmpC-producing and one potential carbapenemase-producing *E. coli* isolate per faecal/swab sample was selected

A2.3.3.2 *Salmonella*

For pig faecal samples, *Salmonella* was isolated by inoculating 100ul of the faecal mixture used for commensal *E. coli* in BPW into 10ml of Rappaport-Vassiliadis (RV) Broth for 18-22 hours at 42°C. The RV broth was then inoculate onto *Salmonella* Brilliance agar and Xylose Lysine Dextrose agar (XLD) (ThermoFisher Scientific), incubated at 37°C and examined after 18-22 hours. Selected colonies were sub-cultured to obtain a pure culture prior to identification by MALDI-TOF MS.

For chicken samples, the above isolation method was followed except that drag swabs (https://www.weberscientific.com/drag-swabs-poultry-sampling-kits-solar-biologicals) were used instead of faeces. Each drag swab was then placed in a sterile Whirlpak® bag (Nasco-Modesto) and 100 mL of buffered peptone water (BPW) (Oxoid) was added. Each sample was gently massaged to mix the contents, drag swab removed, and then incubated at 37°C for 24 hours before inoculating in RV broth as above.

Serotyping of all isolates was performed using slide agglutination and for some isolates by gene sequencing. Only one isolate was selected from each sample.

A2.3.3.3 *Campylobacter* spp.

Campylobacter spp. was isolated from pig and chicken samples by centrifugation of 1ml of faecal BPW mixture at 2000g, and inoculating the soft pellet onto Preston *Campylobacter* agar. Agar was incubated at 42°C under microaerophilic condition for 48 hours. One *Campylobacter* spp. colony was selected from each sample. Selected colonies were subcultured on sheep blood agar for further 24-48 hours prior to identification by MALDI-TOF MS.

A2.3.3.2 *Enterococcus* spp.

Indicator *enterococci* from pigs and chicken were isolated by centrifugation of 1ml of faecal BPW mixture at 2000g, and inoculating the soft pellet onto Slanetz Barrtley agar. The agar was incubated at 42°C with 5% CO2 for 48 hours. Colonies that resemble *Enterococcus* colonies were subcultured for identification. *E. faecium* and *E. faecalis* were selected in both pig and chicken samples. In addition, *E. avium* was also selected in chicken samples (results not shown in report). No more than one isolate for each *Enterococcus* spp. was selected for each sample.

A2.3.4 Susceptibility testing

Identity of isolates from pigs and chicken was confirmed in the bacteriology laboratory using MALDI-TOF MS. Antimicrobial susceptibility testing of all the isolates was performed as minimum inhibitory concentration (MIC) determination using broth microdilution by Sensititre (Trek Diagnostic Systems Ltd.). Inoculation and incubation procedures were in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA]. Result interpretation were in accordance with CLSI and EUCAST [European Committee on Antimicrobial Susceptibility Testing] standard if not available from CLSI. The relevant quality control strains were used at the laboratory: *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Campylobacter jejuni* ATCC 33560, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853.

A2.3.5 MIC plates

For *Enterococcus* spp. CMV4AGP plate and for *Campylobacter* spp. CAMPY plate were used. A customized plate was customized for *E. coli* and *Salmonella* and other fish isolate MIC determinations. Until this plate was available a combination of CMV3AGNF and EURGNCOL plates were used (see tables in Section 3 and Annex 1 for AMs covered).

A2.3.6 Whole genome sequencing and assembly

The DNA of bacterial isolates were extracted using the EZ1 Advanced XL (QIAGEN). The concentration of the DNA were assessed using a Qubit® 2.0 fluorometer, followed by library preparation using Nextera XT library (Illumina). Sequencing was performed on an Illumina MiSeq platform using the MiSeq v3 reagent kit (Illumina) with 2 × 300 bp. Sequencing data were trimmed using Trimmomatic v0.39 (Bolger et al. 2014). De novo assembly was performed using SPAdes version 3.14.1 (Bankevich et al. 2012). Ragout v2.3 (Kolmogorov et al. 2014) was utilized for reference assisted scaffolding

of contigs against two closely related genomes identified using Kmerfinder 3.1 (Hasman et al. 2014) and the PATRIC Similar Genome Finder tool (Wattam et al. 2014) for each isolate. The resulting assembly was polished with Pilon version 1.23 (Walker et al. 2014) and annotated using Prokka version 1.13.3 (Seemann 2014).

A2.3.7 Antibiotic resistance genes detection

CGE ResFinder 4.1 was used to identify antimicrobial resistance genes in the assembled genomes using the database version 2021-04-20 (Zankari et al. 2012). The minimum percentage of the gene length detected and the identity threshold was set to be a 90.0% identity for a positive match. Antimicrobial resistance associated with point mutations were detected using PointFinder 3.1 and the database version 2021-02-01 (Zankari et al. 2017).

A2.3.8 MLST analysis

MLST analysis was performed using BLAST searches against MLST databases derived from http://pubmlst.org/, using mlst tool (http://github.org/tseemann/mlst).

A2.3.9 Plasmid analysis

PlasmidFinder 2.0 was used to identify the presence of plasmids in the draft genomes (Carattoli et al. 2014). Identification was based on the detection of replicon sequences belonging to several known plasmid incompatibility (Inc) groups. The threshold for identification was set to 95% identity and 80% minimum coverage. In addition, PlasFlow was used to distinguish the contigs into plasmid- and chromosome-derived sequences with the default probability threshold of 0.7 (Krawczyk et al. 2018).

A2.3.10 In silico serotyping

Serotype prediction of the strains was carried out using SerotypeFinder 2.0.1 and database version 2019-02-27 based on a threshold of 85% identity and minimum coverage of 60% (Joensen et al. 2015).

A subsample of isolates was selected for gene sequencing based on findings from AM susceptibility testing. Special attention was paid to organisms with resistance to highest priority critically important AMs or resistance to a wide range of AMs.

A2.3.11 Day-old chicks

Samples were collected from day old chicks collected from the hatchery and transported to the laboratory in sterile containers. Chicks were euthanized by

decapitalization and gut samples collected for culture using methods as described earlier.

A2.3.12 Gut contents from day-old chicks for DNA extraction and metagenomics sequencing

Gut samples from day-old chicks were collected for metagenomic analysis for detection of resistance genes. After becoming sedate with chloroform, chicks were euthanized by decapitalization. All stool samples from cecum (no tissue included) were scraped from cecum. DNA from stool samples was extracted by using QIAamp PowerFecal Pro DNA Kit as per manufacturer's instruction. All DNA samples were sent to the Beijing Genomics Institute (BGI) for shotgun metagenomic sequencing as previously described (Fang et al. 2018). In brief, 1 g of genomic DNA was randomly fragmented by Covaris. The fragmented genomic DNA was then selected by Agencourt AMPure XP-Medium kit and those fragmented genomic DNA with an average size of 200-400bp were used to construct the library. Subsequently, the qualified sequencing library was subjected to 100bp paired-end sequencing using the BGISEQ-500 platform.

A2.3.13 Bioinformatics analysis

High-quality reads were obtained by Fastp with default parameters, and were then subject to KneadData (https://huttenhower.sph.harvard.edu/kneaddata/) to remove those belonging to the chicken host genome (Gallus gallus GRCg6a, downloaded from NCBI). MetaPhlAn 3.0 and ARGs-OAP v2.0 were adopted for microbial community and ARGs annotation respectively. Afterward, non-host reads were de novo assembled using SPAdes v3.14.0 with k-mer lengths 21,33,55,77. The open reading frames (ORFs) on assembled contigs predicted by Prodigal v2.6.3 were aligned against SARG v2.2 database (evalue: 1e-5; identity: 0.7; coverage: 0.8) using BLASTX to search ARGs. The ARGs-carried contigs were then aligned to NCBI nt (for nucleotide sequences) databases for taxa identification, in combination with CAT annotation.

A2.4 Antimicrobial usage – fish

A2.4.1 AMU data collection

Fish farmers reported antimicrobial usage (if any), and relevant information such as the category and dosage of antimicrobials administered were captured in a survey form by AFCD representatives during farm visits. Anonymised data on treatment/prescriptions from ambulatory veterinary services were also obtained.

A2.4.2 AMU data analysis

AMU data were collated and recorded in an excel spreadsheet in standardized format to facilitate subsequent AMU calculation. The relevant data recorded for AMU calculations included the active ingredient, antimicrobial class, reported usage in mg, and concentration in mg/kg. The resulting usage metrics include mg of active AM ingredient, mg/kg TAB (target animal biomass), and DDDvet. For the calculation of mg/kg TAB, reference was made to fish farm stocking records.

A2.4.3 AMU audit testing - sampling

Fish, feed, water and sediment samples were obtained from mariculture and pond fish farms/environments and tested for antimicrobial residues. For fish samples, over 500g of the sample (either whole fish or in portions depending on the size) was placed in a labelled and sealed plastic bag. For feed samples, approximately 200g of fish feed was placed in a labelled and sealed plastic bag. For water samples, 500mL of water was collected by submerging a 500mL labelled sterile water bottle not less than 5cm below the water surface. For sediment samples, 200g of sediment were collected using a grab sampler (e.g. Ponar/Ekman grab). Samples were transferred directly onto a sample tray and subsequently into a 200g labelled sterile bottle using a clean spatula. All collected samples were immediately stored in an icebox before being transported to the laboratory for storage at 4°C.

A2.4.4 AMU audit testing - analysis

Samples were dispatched tested for antimicrobial residues by an outsourced laboratory. Samples were extracted using liquid-liquid extraction and subsequently cleaned by solid phase extraction method. The sample solution was analysed by ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Antibiotics residues were extracted into water and acetonitrile mixture with the aid of Ethylenediaminetetraacetic acid (EDTA) disodium salt by shaking and sonication. Resulting aqueous sample solution was passed through C18 solid phase extraction tube for purification. The final sample solution was subjected to UPLC-MS/MS analysis.

A2.5 Antimicrobial resistance – fish

A2.5.1 AMR sample collection

Slime swabs for AMR testing were collected off fish samples obtained from 20 pond fish farms and 96 mariculture farms. Sediment and water samples were also collected from marine waters and ponds for AMR testing.

For fish samples, surface slime of skin and gills were swabbed (one swab per fish) using sterile transport swabs and subsequently sealed in plastic bags. Collected samples were stored in an ice box at 4°C and transported to the laboratory. Water and sediment samples were collected and stored using the methodology described above in "AMU audit testing".

A2.5.2 Culture for bacterial isolates

For pond fish samples, fish slime swabs were streaked on Blood Agar (BA), 0% Trypticase Soy Agar (TSA) and 2% TSA, while for marine fish samples, fish slime swabs were streaked on BA, 2% TSA, 5% TSA and Thiosulfate-citrate-bile-salts-sucrose (TCBS) Agar and then incubated at 21-29°C for 24-48 hours. Typical colonies were selected and screened for indicator microorganisms of Aeromonas spp. for pond fish, and Photobacterium spp. and Vibrio spp. for marine fish.

Water samples were filtered using a sterile 0.45 µm membrane using a membrane filtration method and transferred the filtered membrane into a sterile tube with Alkaline Buffered Peptone (for pond water samples) or Buffered Peptone (for marine water samples). Tubes were incubated at 29°C for 24 hours and screened for indicator microorganisms following the same methodology used for fish slime swabs.

Sediment samples were transferred onto a sterile tube with Alkaline Buffered Peptone (for pond sediment) or Buffered Peptone with 3% salt (for marine sediment). Tubes were incubated at 29°C for 24 hours and screened for indicator microorganisms following the same methodology used for fish slime swabs.

The identification of bacterial isolates was confirmed using MALDI-TOF.

A2.5.3 Antimicrobial susceptibility testing

Antimicrobial susceptibility of bacterial isolates was determined by minimum inhibitory concentration (MIC) testing, and this was performed against 29 antimicrobials dehydrated on both CMV4AGNF and HKGAFCDDF Customized 96-well microplates using broth microdilution by Sensititre (Trek Diagnostic Systems Ltd.). Inoculation and incubation procedures were in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA]. The relevant quality control strains of *Escherichia coli* ATCC 25922 was used in parallel to the MIC testing.

A2.5.4 Whole genome sequencing and assembly

Whole genome sequencing was performed on selected bacterial isolates. DNA was extracted using DNeasy PowerSoil Kit (QIAGEN), and the concentration of the DNA were assessed using a Qubit® fluorometer (Thermo Fisher) and Nanodrop microspectrophotometer (Thermo Fisher). Extracted DNA was sent for high-throughput metagenomics sequencing using Illumnia Hiseq4000 platform with PE150 strategy at Novogene Co. Ltd. For Nanopore long-read sequencing, library preparation was performed using SQK-RBK004 rapid barcoding sequencing kit following the protocol. Nanopore sequencing was conducted using R9.4 flow cells. High-quality genomes were of each bacterial isolate were constructed by Unicycler (Wick et al. 2017), and the completeness and contamination of hybrid-assembled genomes were evaluated by CheckM (Parks et al. 2015). The taxonomy of the assembled genomes were classified by GTDB-Tk (Chaumeil et al. 2019) and the genome annotation was conducted using Prokka (Seemann 2014).

A2.5.5 Antibiotic resistance genes detection

The annotated 16S rRNA genes in the genomes of each isolate were extracted and blasted against the NCBI 16S rRNA gene database, and the five most similar 16S rRNA were retrieved to construct the phylogenetic tree. Then ARGs were determined by BLASTp predicted ORFs (open reading frames from Prokka) against ARGs amino acid database (SARG2.2) developed with the cut-off: E value of 10-5, 70% coverage, and 80% similarity (Yin et al. 2018). Finally, the whole genome arrangement including the ARGs arrangement was visualized using cgview (Grant and Stothard 2008).

A2.5.6 Plasmid analysis

For plasmids, PlasFlow (Krawczyk et al. 2018) was used to predict plasmid sequences from the hybrid assembled contigs.

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