



One Health Report on **Antimicrobial Utilisation and Resistance, 2017**

One Health Antimicrobial Resistance Working Group,
Singapore

One Health Report on Antimicrobial Utilisation and Resistance, 2017

An overview of national surveillance programmes on antimicrobial utilisation and resistant organisms up to 2017.

A Report of the One Health Antimicrobial Resistance Working Group

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Participating ministries and agencies¹:



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Enquiries:

Enquiries regarding this report should be addressed to:

AMR Coordinating Office, NCID

Email: narcc_sec@ncid.sg

¹ With effect from 1 April 2019, the Agri-Food and Veterinary Authority (AVA) was restructured into the Singapore Food Agency (SFA) and the Animal & Veterinary Service of National Parks Board (NParks/AVS). SFA will continue the current work on the food-related aspects of AMR, while NParks/AVS will focus on the animal health-related aspects of AMR.

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The One Health Antimicrobial Resistance Workgroup wishes to thank the following contributing authors:

- A/Prof Lim Poh Lian, NARCC Chair, MOH
- Ms Lin Yueh Nuo, NCID
- Mr Chan Guan Hao, MOH
- Dr Kelvin Lim, AVA
- Dr Shawn Ting, AVA
- Ms Wong Wai Kwan, AVA
- Ms Tan Wei Ling, AVA
- Dr Brian Tan, AVA
- Dr Aung Kyaw Thu, NEA
- Dr Chang Siao Yun, PUB
- Dr Martin Tay, PUB

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Note: In 1 April 2019, the Agri-Food and Veterinary Authority (AVA) was restructured into the Singapore Food Agency (SFA) and the Animal & Veterinary Service of National Parks Board (NParks/AVS). SFA continues the current work on the food-related aspects of AMR, while NParks/AVS will focus on the animal health-related aspects of AMR. This report was first compiled in March 2019 as a reference for the One Health agencies, and reflects the organisations involved, their contributions to national AMR surveillance and authors' affiliations at the time of writing.

² Renamed the Veterinary Diagnostic and Research Laboratory, w.e.f. 1 April 2019

³ Renamed the National Centre of Food Science, w.e.f. 1 April 2019

Executive Summary

Antimicrobial-resistant bacteria can be found in hospital settings, community, animals, food products and environments (both built and natural) worldwide. While antimicrobial resistance (AMR) is a naturally-occurring phenomenon, its development is often accelerated by the misuse and overuse of antimicrobials in humans and agriculture. Trends in the levels and types of resistance, and in the quantities of antimicrobial utilisation (AMU) therefore need to be monitored at national and global levels to provide scientific data for guiding policy decisions and measuring the impact of interventions.

Previously, surveillance activities in Singapore had been reported independently by different ministries and agencies for the sectors under their purview. A key initiative of the National Strategic Action Plan on AMR was therefore to unify these existing independent efforts. To support this initiative, the AMR Coordinating Office (AMRCO) was established in 2018 to ensure that the collection and analysis of surveillance data was coordinated across the human, animal, food and environment sectors. This first multi-sectoral report provides an overview of the main national surveillance activities conducted in the human, animal, food and environment sectors up to the end of 2017. The objective going forward is to build a more integrated national surveillance system for AMR and AMU that can inform on priority pathogens and antimicrobial utilisation across all sectors and apply the data to risk assessment and control measures for AMR.

In compiling this report, we found that sectors targeted different organisms for surveillance according to sector priorities and concerns. Few antibiotic-bacteria pairings were prioritised by all four sectors. Nevertheless, several common concerns were revealed, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta-lactamase (ESBL)-producing *E. coli*. These present potential areas for deeper collaboration and integration across sectors.

Current gaps in AMU surveillance include the scarcity of data on antimicrobial consumption in the private sector and community, and on total national consumption data that would be useful for international benchmarking and comparison. To this end, we are exploring ways to collect meaningful data from these sectors in a reliable and sustainable manner, and to support global efforts in AMR and AMU data collection by WHO. In the animal sector, establishing systems to collect farm-level AMU data, improving wholesaler data reporting and expanding AMR surveillance to all key food-producing animals are our next priorities in strengthening the knowledge base.

The key findings for each sector are summarised below.

HUMAN

The National Antimicrobial Resistance Control Committee (NARCC) has been monitoring antimicrobial resistance and utilisation in public hospitals since 2011. NARCC advises the Ministry of Health (MOH) on the development of strategies and policies to control the emergence and spread of AMR in Singapore. NARCC also monitors antimicrobial stewardship programmes (ASPs), which have been

established and funded in public hospitals since 2011. NARCC also reports on appropriate carbapenem initial prescribing rates, number of antibiotic audits conducted, and acceptance of ASP advice for each hospital.

From 2011 to 2017, fluoroquinolones, particularly oral ciprofloxacin, were the most heavily used antibiotics, with usage up to approximately 128 g per 1,000 inpatient-days, followed by broad spectrum penicillins, cephalosporins, and carbapenems.

In addition to a range of resistant bacteria causing human infections, NARCC monitors three key AMR indicators, to encourage hospital accountability for patient safety and care. Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia decreased by 13% per 10,000 inpatient days in 2017 compared with 2016, and *Clostridium difficile* detection rates decreased by 10% over the same period. The latter is a marker of total antibiotic use burden, and its reduction reflects positively on hospitals' efforts in enhancing antimicrobial stewardship and infection prevention. Carbapenamase-producing Enterobacteriaceae (CPE) clinical decreased by 9% from 2016 rates, while CPE screening isolates increased by 77%, likely a result of increased screening. CPE is a major concern in human health because carbapenems are considered antibiotics of last resort. Resistance to carbapenems has been spreading between bacterial species, and can be transmitted among patients. Increase in screening isolates for CPE reflect active surveillance by hospitals to identify carriers early so that enhanced infection control measures can be undertaken to reduce such cases.

ANIMAL

Total antimicrobial consumption (in total kg) in the agriculture sector in Singapore is relatively low due to the limited agriculture here. Singapore follows the World Organisation for Animal Health (OIE)'s template for reporting data on different antimicrobial classes and for different animal sectors. Tetracyclines, fluoroquinolones and penicillins were identified as the top three antimicrobial classes of drugs used for veterinary purposes from 2015 to 2017 in Singapore, based on a survey of local veterinary drug wholesalers, of which approximately 50% participated. The aquaculture industry was the main consumer of antimicrobials, whereas consumption in terrestrial food-producing animals was relatively low.

Routine monitoring for AMR organisms is prioritised to detect multi-drug resistant (MDR) *Salmonella* species in local poultry farms. The average annual prevalence of MDR *Salmonella* in local chicken layer farms remained low (3.3%), while overall prevalence in local quail layer farms decreased from 37% in 2010 to 4% in 2016 and 8% in 2017. The *mcr-1* gene conferring colistin resistance was infrequently found in *Salmonella* isolates from local farms (0.03%). Livestock-associated-MRSA (LA-MRSA) was found in 38.3% of pigs at the abattoir, of which 90% were sequence type 9 (ST9), characteristic of Asian-origin LA-MRSA, and 10% were ST398. In companion animals, methicillin-resistant *Staphylococci* were isolated from 3.6% of samples tested from 2014 to 2017; 90% of isolates from 2017 were identified as methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), a species more commonly identified in animals than humans.

FOOD

The food chain is a potential route for transmission of resistant bacteria and resistance genes. *Salmonella* species, as the leading cause of foodborne illness in Singapore, is a priority organism for control at both import and retail levels. High levels of multi-drug resistance were found among *Salmonella* species isolated from imported poultry and poultry meat: 82.3% of isolates from frozen chicken from South America and 54.7% of isolates from Malaysian fresh chicken were MDR, defined as being resistant to three or more classes of antimicrobials. However, these higher MDR rates should be taken in context of the relatively low rates of *Salmonella* contamination, which is approximately 3.2% in frozen poultry at point of import and 9.4% in freshly slaughtered carcasses. Furthermore, import controls are placed on consignments and farms contaminated with *Salmonella*, particularly with *Salmonella enterica* serovar Enteritidis, which is the leading cause for non-typhoidal salmonellosis in Singapore. MDR was found to be highest among *S. enterica* ser. Albany and *S. enterica* ser. Brancaster, and lowest among *S. enterica* ser. Enteritidis and *S. enterica* ser. Kentucky. Although infrequently detected, *Salmonella* isolates resistant to at least one antimicrobial was found in raw food (26.3% of isolates), cooked/ready-to-eat food (38.9%) and in humans (38.2%). Isolates were resistant to tetracycline and ampicillin, which are antibiotics commonly used in agriculture, and less often to ceftriaxone and ciprofloxacin, which are antibiotics frequently used for the treatment of salmonella in human health.

MRSA, vancomycin-resistant Enterococcus (VRE) and ESBL-producing *E. coli* detection rates were generally low in target foods sampled at points of import. MRSA was found in <1% of raw imported meat products tested, VRE (mostly *Enterococcus durans*) in <5% of slaughterhouse poultry viscera and ESBL *E. coli* in approximately 5% of samples tested. The *mcr-1* gene conferring resistance to colistin was detected in 18.4% of *E. coli* found in imported food products from 2014 to 2016, of which 11% were also resistant to beta-lactam antibiotics. However, the gene was less frequently detected in *Salmonella* spp. food isolates (1.25%).

At the retail end of the food chain, *E. coli* and *K. pneumoniae* isolates obtained from raw, cooked and ready-to-eat food products were found to be 24% and 5.2% MDR respectively. Of the retail *E. coli* isolates, 2% were ESBL-producing. Of 227 *S. aureus* isolates from cooked/ready-to-eat retail food products and food contact surfaces (e.g. gloves), 1.8% were MDR strains and 2.2% were MRSA of sequence types ST80 and ST6. Based on the phenotypic and genotypic characteristics, MRSA isolates from retail food products were identified as community-associated (CA-MRSA) strains, which are more likely associated with human, rather than animal, origin. There is so far, no evidence of livestock-associated MRSA (LA-MRSA; ST398 and ST9) in retail food products.

These results highlight the importance of reinforcing safe food practices and good personal hygiene to reduce exposure to pathogens and drug-resistant organisms in food.

WATER ENVIRONMENT

Urban waters present a potential environment for the exchange of resistance determinants. Enterobacteriaceae were the predominant bacteria isolated from hospital and domestic wastewaters. These waters also contained comparably high levels of faecal indicator bacteria loads, which were found to be most effectively reduced by the advanced Membrane Bioreactor treatment process.

Human activity has impact on the levels of resistant bacteria in the water environment. A study on local domestic and hospital wastewaters, reservoirs and catchments showed that hospital wastewater had the highest occurrence of antibiotic resistant bacteria and antibiotic resistant genes. Furthermore, a local watershed, defined as an area of land that feeds all the water above and underground into a waterbody, located in an urbanised landscape was found to contain more resistant bacteria than a watershed that receives water from a mixture of forested and less urbanised landscapes. However, the presence of bacteria in watersheds is mitigated by effective water treatment systems in place in Singapore. Data from environmental surveillance of AMR bacteria and genes will be used to assess the risk of AMR in urban waters.

Introduction

The Singapore National Strategic Action Plan on Antimicrobial Resistance (AMR) was jointly developed by the Ministry of Health (MOH), Agri-Food and Veterinary Authority (AVA), National Environment Agency (NEA) and PUB, the national water agency, and launched on 1 November 2017. The Plan aims to reduce the emergence and prevent the spread of drug-resistant organisms through five core strategies: Education, Surveillance and Risk Assessment, Research, Prevention and Control of Infection and Optimisation of Antimicrobial Use.

Surveillance on AMR trends in specific organisms and antimicrobial utilisation (AMU) is important to provide data for risk assessment, monitoring trends and guiding policy decisions. Such data are useful for measuring outcomes of programmes and initiatives and for assessing the overall impact of the National Strategic Action Plan for AMR.

Prior to the launch of the National Strategic Action Plan, surveillance and monitoring of AMR and AMU in Singapore had been conducted and reported independently by different ministries and agencies for the sectors under their purview. However, the nature of the AMR threat demands a concerted “One Health” approach requiring action from all relevant sectors and stakeholders. Hence, a key initiative under the Action Plan is to unify the existing independent efforts and establish a system to integrate surveillance for AMR and AMU in the human, animal, food and environment sectors, and share information across sectors. This will help all parties involved to better understand the extent and interdependencies of this complex issue.

To support this initiative, the Ministry of Health established the Antimicrobial Resistance Coordinating Office (AMRCO) in 2018 under the auspices of the National Centre for Infectious Diseases (NCID). One of the key roles of the Office is to facilitate the sharing of data between sectors, and ensure that the collection and analyses of surveillance data across human, animal, food and environment sectors are coordinated.

This first joint surveillance report on AMR and AMU aims to provide an overview of surveillance and monitoring activities carried out by the national ministries and agencies up to the end of 2017 and inform of the key findings from these programmes. This joint report serves as a starting point towards a more integrated approach for national AMR and AMU surveillance across the human, animal, food and environment sectors in Singapore.

PART I.

ANTIMICROBIAL UTILISATION

Antimicrobial Utilisation in Humans

NATIONAL ANTIMICROBIAL RESISTANCE CONTROL COMMITTEE

MOH appointed the National Antimicrobial Taskforce (NAT) in August 2009 to address the problem of AMR in Singapore. In 2011, MOH approved NAT's recommendations for surveillance of AMR, antimicrobial utilisation and stewardship (AUS) in hospitals.

In 2014, NAT was re-constituted as a standing committee, the National Antimicrobial Resistance Control Committee (NARCC), to assist MOH in developing strategies to control the emergence and spread of AMR in Singapore. NARCC plays a critical role in recommending and driving initiatives related to the control of AMR in Singapore and is supported by two expert panels: the National Antimicrobial Resistance Expert Panel (NAREP), and the National Antimicrobial Stewardship Expert Panel (NASEP). The expert panels, comprising microbiologists, infectious disease (ID) physicians and pharmacists, working closely with public health colleagues, assist the Committee on issues related to surveillance of antimicrobial-resistant organisms and monitoring of AUS.

ANTIMICROBIAL STEWARDSHIP PROGRAMME

Antimicrobial stewardship programmes (ASPs) were implemented in Singapore to promote judicious and appropriate use of antimicrobials in all public hospitals for effective control of AMR. Since 2011, MOH has provided funding for manpower including ID physicians and pharmacists, as well as the development of computerised decision support systems (CDSS) to support ASPs, monitor and improve antimicrobial prescribing in the public hospitals. ASP teams conduct audits on antimicrobials used, provide feedback to prescribing clinicians, collate rates of appropriate initial carbapenem prescribing, and acceptance rates of ASP advice given. Through this programme, ASP pharmacists and ID specialists work closely with various teams in the public hospitals to develop guidelines for appropriate use of antimicrobials to prevent the emergence of AMR.

ANTIMICROBIAL UTILISATION AND STEWARDSHIP DATA

As part of NARCC's initiatives on antimicrobial stewardship and the control of AMU, NASEP monitors AMU in the public hospitals on a six-monthly basis, using defined daily doses (DDD) per 1,000 inpatient days. DDD is the average daily maintenance dose for a drug's main indicated use in adults and is a standard determined by the World Health Organization (WHO). Although DDD has certain limitations, it is a commonly accepted and practical way to measure antimicrobial consumption. Figures 1 to 4 show the total utilisation of the four most important antibiotic groups in public hospitals: broad-spectrum penicillins, 3rd & 4th generation cephalosporins, fluoroquinolones (oral ciprofloxacin) and carbapenems, respectively. Fluoroquinolones, particularly oral ciprofloxacin, were the most heavily used antibiotics, with usage up to approximately 128 g per 1,000 inpatient days, followed by broad

spectrum penicillins, cephalosporins, and then carbapenems. Of the four most important antibiotic groups, there has been a decline of fluoroquinolone and 3rd & 4th generation cephalosporins use in public hospitals since 2011, but an increase in broad-spectrum penicillins (amoxicillin/clavulanate and piperacillin/tazobactam). Use of carbapenems have remained generally stable. With more data over time, it may be possible to correlate AMU with AMR trends, and to measure the impact of antimicrobial stewardship initiatives.

By reducing antimicrobial pressure, NASEP aims to reverse the rising tide of AMR, and its adverse impact on patients, healthcare resources, manpower and expenditure.

Figure 1: Utilisation of broad-spectrum penicillins in public hospitals

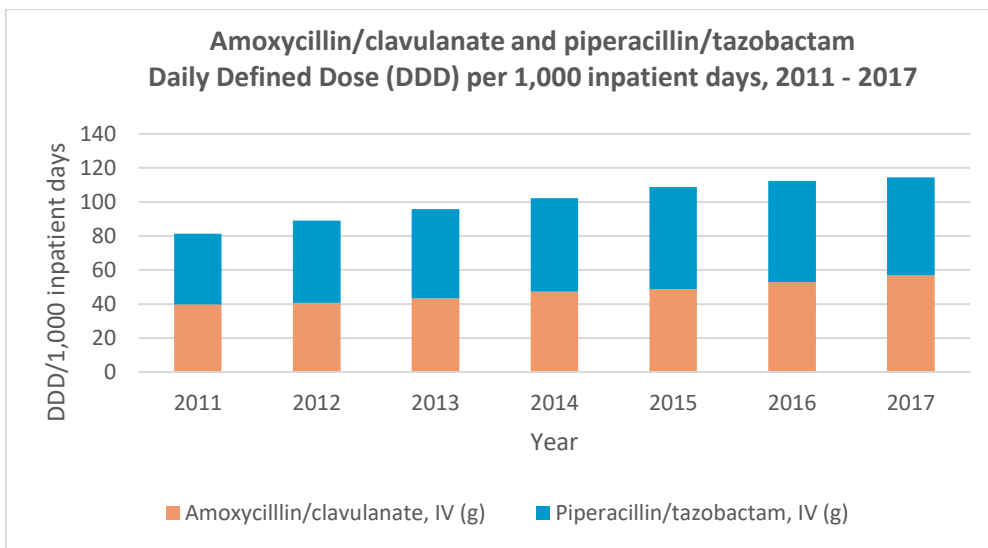


Figure 2: Utilisation of 3rd and 4th generation cephalosporins in public hospitals

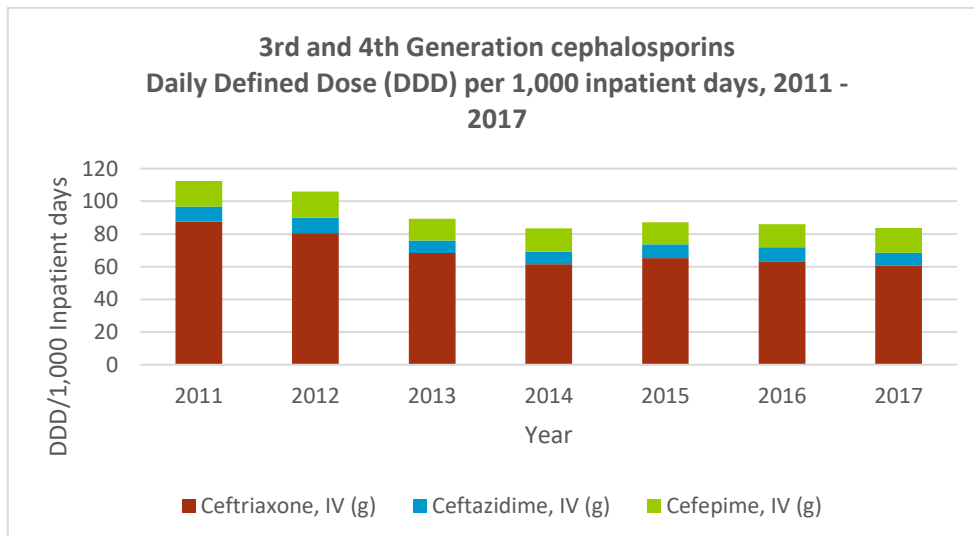


Figure 3: Utilisation of fluoroquinolones (oral ciprofloxacin) in public hospitals

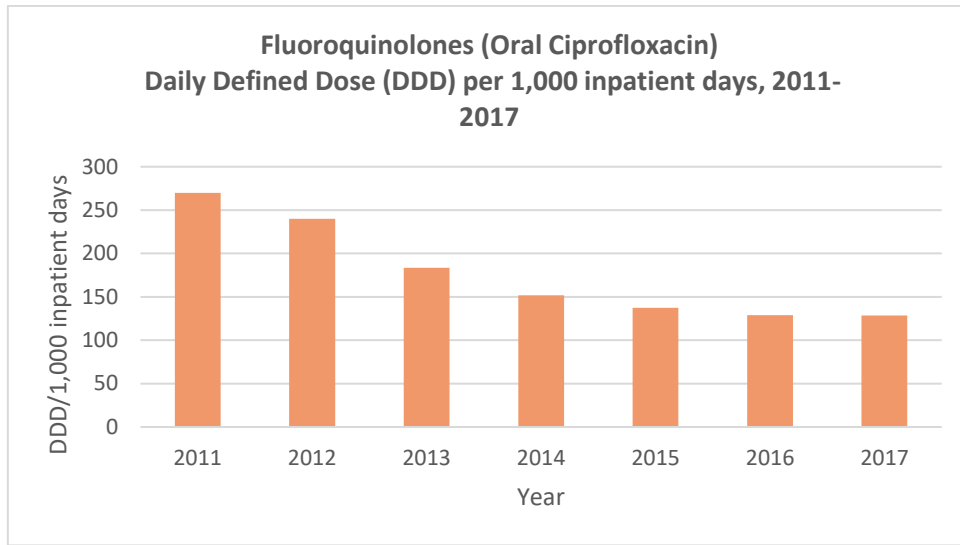
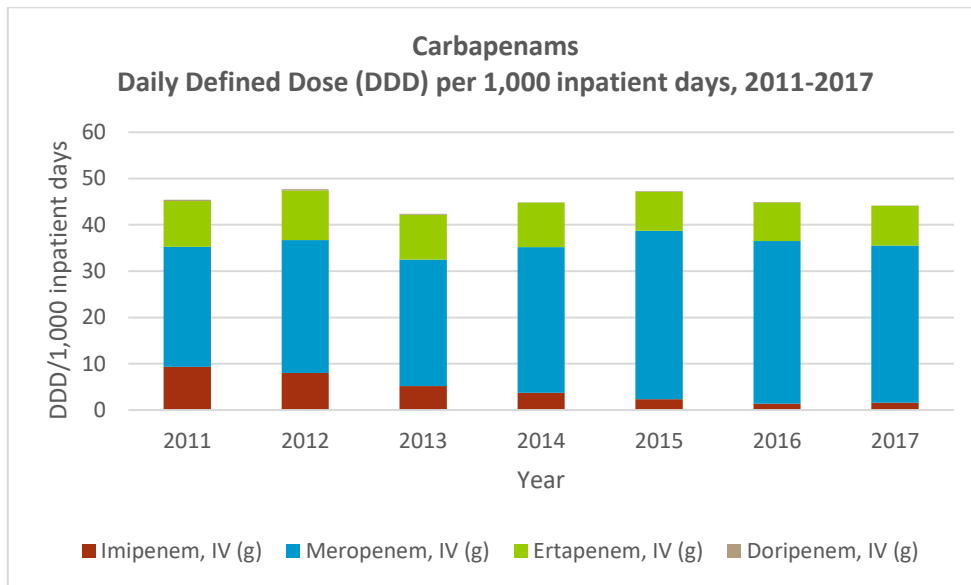


Figure 4: Utilisation of carbapenems in public hospitals



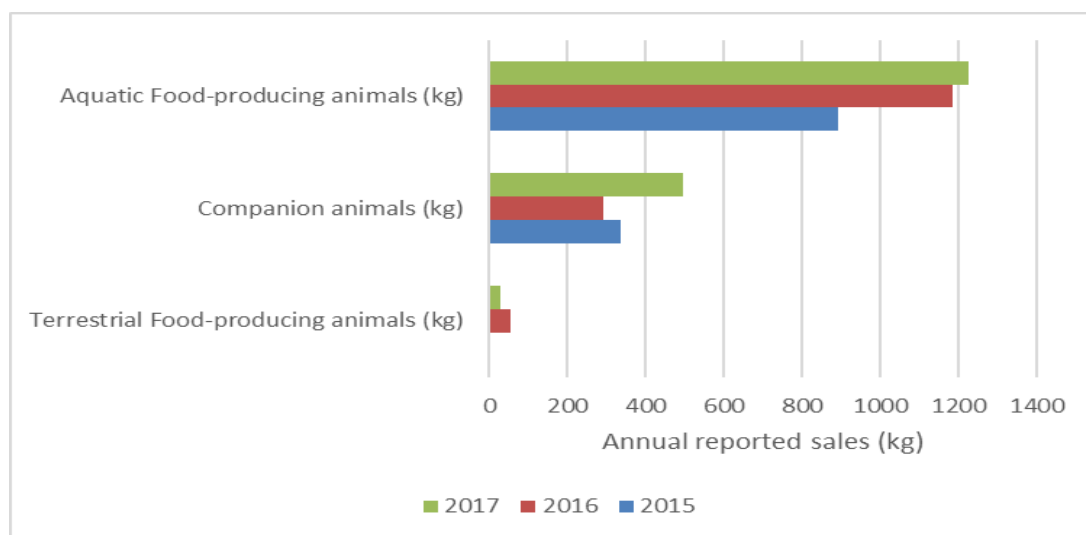
Antimicrobial Utilisation in Animals

Chicken layer and food fish farms are the most significant food animal-producing farms in Singapore. There are 3 layer farms that together house over 2 million chickens. These farms produce about 27% of the total egg consumption in Singapore. In addition, 126 fish farms (of which 9 are land-based) produce approximately 10% of total local fish consumption.⁴ The remaining food animal population is made up of approximately 123,000 quail layers, 170 dairy cattle and 540 dairy goats.

In Singapore, antimicrobials are allowed for use in the treatment and prevention of diseases in animals. Antibiotics are not allowed for growth promotion⁵. The AVA also prohibits the use of certain substances such as chloramphenicol, avoparcin, beta-agonists and nitrofurans, in local food-producing animals as well as in animal feed. The use of antimicrobials in local chickens throughout the laying period is also disallowed to prevent the presence of residues in eggs.

Data on AMU in animals has been collated and reported annually to the World Organisation for Animal Health (OIE) since 2015. Currently, sales data is collected through a voluntary survey of wholesalers of veterinary drugs and serves as a proxy for antimicrobial utilisation in animals. Figure 5 shows the breakdown of reported sales to the different animal sectors from 2015 to 2017, collated from responses of 52% (12 of 23), 54% (14 of 26) and 54% (13 of 24) of wholesalers respectively. The annual reported sales of antimicrobial drugs for veterinary therapeutic use (including the prevention of clinical signs) is shown in Figure 6. No antimicrobials were sold for non-therapeutic uses (e.g. growth promotion).

Figure 5: Annual reported sales (kg) of antimicrobial drugs by animal sector, 2015 to 2017.

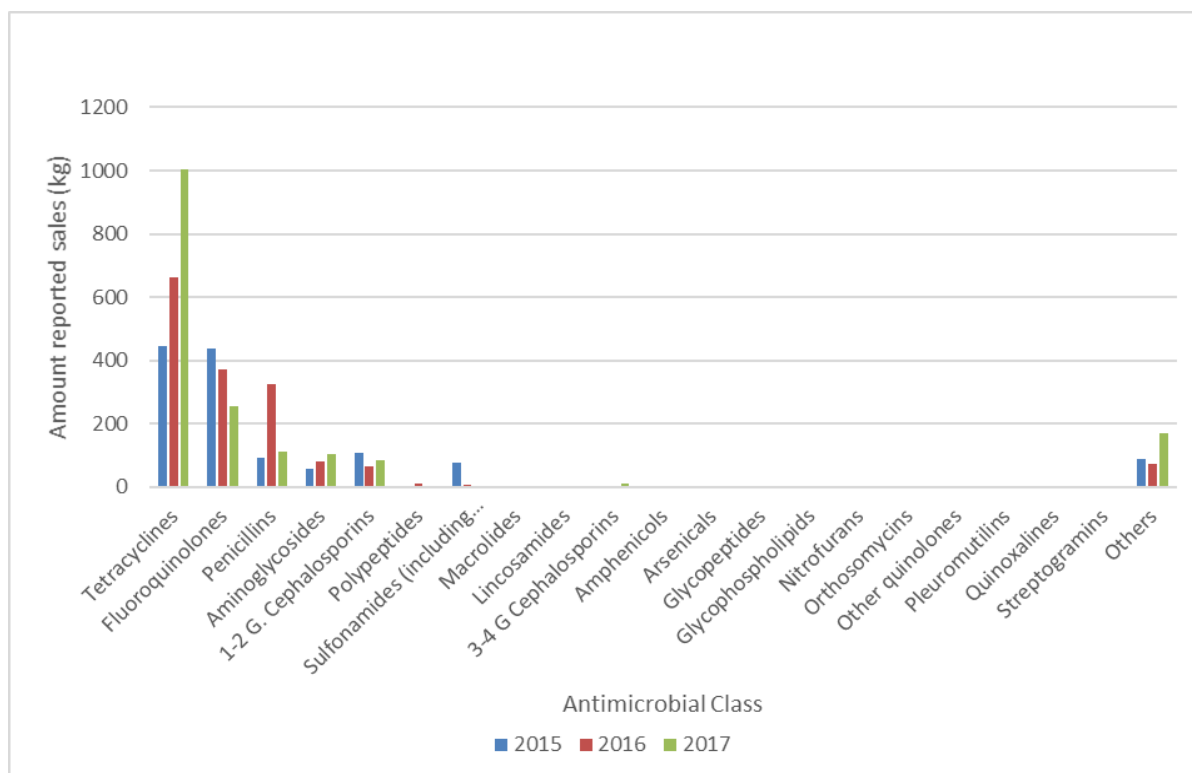


Note: Reported sales to terrestrial food-producing animals in 2015 was <1.0 kg.

⁴ AVA Annual Report 2017-2018

⁵ This includes and is not limited to use of sub-therapeutic doses of an antimicrobial without therapeutic judgement and use of an antimicrobial labelled for growth promotion at the recommended dose for growth promotion.

Figure 6: Annual reported sales (kg) of antimicrobial drugs in the animal sector, by antimicrobial class, 2015 to 2016.



In terms of volume (kg), the aquaculture industry is the largest consumer of antimicrobials in animals in Singapore. Reported sales of tetracyclines increased each year from 2015 to 2017, attributed largely to increased sales to the aquaculture sector. Meanwhile, fluoroquinolone (enrofloxacin) sales decreased yearly from 2015 to 2017. However, the data currently available are insufficient to draw conclusions on the possible reasons for these observed trends.

AMU in terrestrial food-producing animals is low relative to other animal sector. This is despite the highly intensive nature of poultry layer farms in Singapore. This is reflective of the mature state of the poultry layer industry here, characterised by adherence to good animal husbandry practices, effective biosecurity measures to prevent disease introduction, routine vaccination programmes for disease prevention and good compliance to restrictions on AMU in layers.

In the local aquaculture scene, other than a few larger progressive farms, most fish farms here are small holdings. Antimicrobial usage is further compounded by the limited availability of tropical food fish vaccines worldwide, and reduced cost-effectiveness of vaccination for smaller holdings.

Outreach and education, accreditation schemes, good aquaculture practices, facilitation of vaccine registration and use, development of prudent use guidelines and strengthening of regulations are part

of AVA's overall strategy towards a reduction of AMU in the aquaculture industry, especially those critically important to human health⁶.

Conclusion and Next Steps

Antimicrobial utilisation data is comprehensive and closely monitored in Singapore public hospitals, with regular reporting to MOH and individual hospitals through NARCC. At the time of this report, we are working with the private and community sectors to gather AMU data, as well as identifying sources of total national consumption data.

Total antimicrobial consumption (in total kg) in the agriculture sector in Singapore is relatively low due to limited agriculture industry here. Singapore follows the OIE's template for reporting data on different antimicrobial classes and for different animal sectors. However, data collection is currently based on voluntary submission by veterinary drug wholesalers in Singapore, with a participation rate just above 50%. Annual trends in sales are therefore difficult to interpret. At present, there is also no data on farm or community utilisation. Improving wholesaler participation, and establishing systems to collect farm- and community-level data are our next steps in strengthening AMU data collection in the animal sector.

⁶ As listed in Critically Important Antimicrobials for Human Medicine, WHO 2017

PART II.
SURVEILLANCE & MONITORING OF
ANTIMICROBIAL RESISTANT BACTERIA

Antimicrobial Resistance in Public Hospitals

ANTIMICROBIAL RESISTANCE DATA

As part of the initiatives to control AMR, NARCC monitors aggregated data on antimicrobial resistant organism isolates from public hospitals. Rates of resistant organism infections caused by resistant organisms, along with the hospital antimicrobial utilisation data, are presented to the Ministry of Health and to individual hospitals for their monitoring and implementation of appropriate control actions where needed. Screening samples are excluded in most instances. Clinical isolates are counted once in every 6-month period per patient. While clinical isolates may include colonisation, they provide a useful indicator for total AMR burden, including consumption of hospital resources (e.g. isolation rooms, gowns, gloves and manpower). Bacteraemia rates generally represent true infection.

Figure 7 is a graphic representation of the following four key AMR and AMU indicators that provide a quick overview of the AMR and AMU landscape within the public hospitals:

- (i) Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia rates – MRSA is a common AMR organism in healthcare institutions;
- (ii) Carbapenemase-producing Enterobacteriaceae (CPE) clinical isolate numbers – CPE are AMR organisms of importance due to their wide range of resistance;
- (iii) *Clostridium difficile* rates – *Clostridium difficile* infections are associated with antimicrobial overuse;
- (iv) Percentage of appropriate initial carbapenem prescribing – carbapenems are broad spectrum antibiotics reserved for treatment of infections caused by drug-resistant organisms.

Figure 7: Key hospital antimicrobial resistance and utilisation indicators, 2016-2017

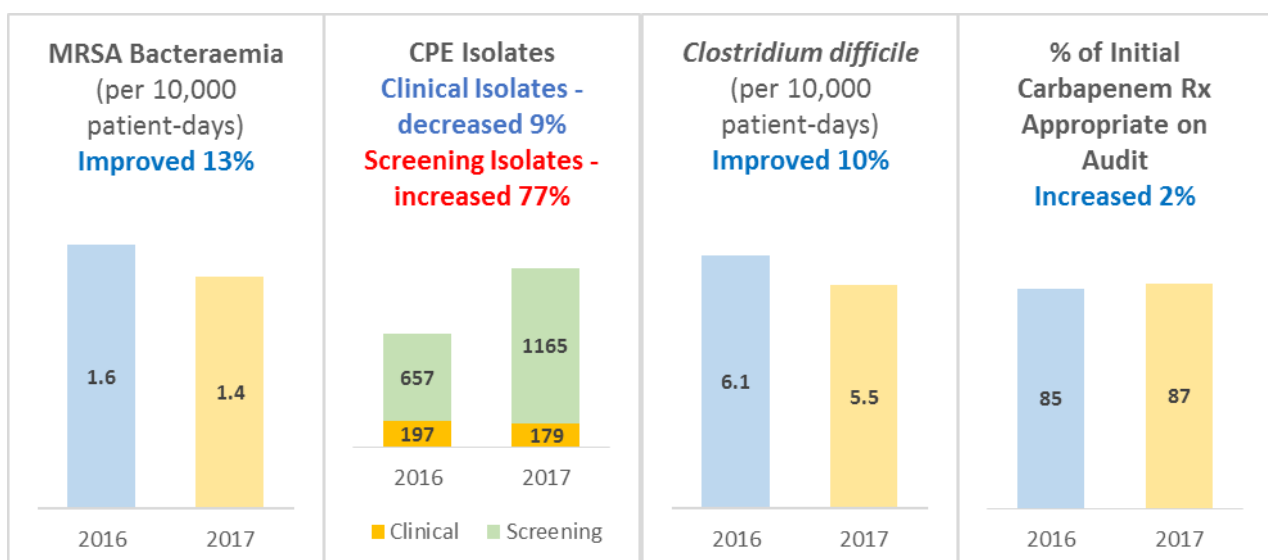


Table 1 compares the number of isolates and the incidence density per 10,000 inpatient days for different organisms between 2016 and 2017, with green indicating improvement, and red indicating worsening resistance. The organism with the highest incidence density per 10,000 inpatient days is *E. coli*, consistent with the overall trend of increasing resistance in Enterobacteriaceae. Table 2 depicts a national antibiogram for WHO priority organisms: 24.6% of *S. aureus* isolates were MRSA, 25.2% of *E. coli* and 28.2% of *K. pneumoniae* isolates were resistant to ceftriaxone, 39.1% of *E. coli* were resistant to ciprofloxacin, and 43.2% of *A. baumannii* isolates were carbapenem-resistant.

Table 1: Comparison of number of isolates and incidence density, 2016 – 2017

Organism	No. of isolates		Incidence density per 10,000 inpatient days	
	2016	2017	2016	2017
Methicillin resistant <i>S. aureus</i>	2,587	2,572	11.5	10.9
Methicillin resistant <i>S. aureus</i> (B)	356	327	1.6	1.4
<i>E. coli</i> , Ceftriaxone resistance	5,703	5,513	25.4	23.4
<i>E. coli</i> , Ceftriaxone resistance (B)	920	869	4.1	3.7
<i>E. coli</i> , Ciprofloxacin resistance	8,357	8,542	37.3	36.2
<i>E. coli</i> , Ciprofloxacin resistance (B)	1,174	1,159	5.2	4.9
<i>E. coli</i> , Meropenem or imipenem resistance	69	69	0.3	0.3
<i>K. pneumoniae</i> , Ceftriaxone resistance	2,806	2,879	12.5	12.2
<i>K. pneumoniae</i> , Ceftriaxone resistance (B)	390	388	1.7	1.6
<i>K. pneumoniae</i> , Ciprofloxacin resistance	3,008	3,061	13.4	13.0
<i>K. pneumoniae</i> , Ciprofloxacin resistance (B)	431	473	1.9	2.0
<i>K. pneumoniae</i> , Meropenem or imipenem resistance	169	169	0.8	0.7
<i>P. aeruginosa</i> , Meropenem or imipenem resistance	754	729	3.4	3.1
<i>P. aeruginosa</i> , Meropenem or imipenem resistance (B)	56	70	0.2	0.3
<i>A. baumannii</i> , Meropenem or imipenem resistance	474	430	2.1	1.8
<i>A. baumannii</i> , Meropenem or imipenem resistance (B)	48	47	0.2	0.2
<i>A. baumannii</i> , MDR resistance	298	290	1.3	1.2
VRE	246	230	1.1	1.0
<i>C. difficile</i>	1,376	1,303	6.1	5.5

(B): bacteraemia

Table 2: Percentage of resistant isolates for WHO priority organisms – 2017

Organism	Methicillin resistance	Ceftriaxone resistance	Ciprofloxacin Resistance	Carbapenem resistance
<i>S.aureus</i>	24.6%	N/A	N/A	N/A
<i>E.coli</i>	N/A	25.2%	39.1%	0.3%
<i>K.pneumoniae</i>	N/A	28.2%	30.0%	1.8%
<i>P.aeruginosa</i>	N/A	N/A	N/A	12.1%
<i>A.baumannii</i>	N/A	N/A	N/A	43.2%

Antimicrobial Resistance in Animals

The AVA Animal Health Laboratory (AHL) has been monitoring the antibiotic resistance profiles of *Salmonella* spp. isolated from local poultry farms under AVA's *Salmonella* Enteritidis (SE) surveillance programme since 2008. Monitoring of antibiotic resistance profiles of *Salmonella* spp. and *Escherichia coli* (*E. coli*) isolated from local poultry and ruminant farms was introduced in November 2017 ; data collection is ongoing.

In 2010, AVA conducted a six-month study to estimate the prevalence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in pigs imported for slaughter. Another study to screen for the presence of the colistin resistance *mcr-1* gene in archived *Salmonella* isolates from local and overseas farm samples was conducted in 2015-2016.

Surveillance in companion animals and zoological specimens is passive and based on clinical isolates. Clinical isolates obtained from samples submitted by veterinarians undergo antibiotic susceptibility testing to guide treatment options. Isolates of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* from companion animals are tested for methicillin resistance, and archived for further characterisation studies.

All antibiotic susceptibility tests (AST) were conducted by disk diffusion according to CLSI standards, applying veterinary breakpoints (CLSI VET001). Bacteria are identified as multi-drug resistant (MDR) if resistant to three or more classes of antibiotics.

Findings from these programmes are summarised below.

RESISTANCE PROFILE OF SALMONELLA IN LOCAL CHICKEN LAYER FARMS

From 2010 to 2017, 739 different *Salmonella* spp. were isolated from Singapore's three chicken layer farms. Twenty-four (3.2%) of the *Salmonella* isolates were MDR. No MDR *Salmonella* was isolated before 2012. However, a spike was observed in 2013 where 8 of 89 (9%) *Salmonella* isolated were MDR (Figure 8), which correlated with a temporary increase in resistance to tetracycline and streptomycin in 2013 (Figure 9). This spike may have been associated with a change in the overseas source of day-old chicks occurring at that time, as local farms are largely dependent on sources overseas for day-old chicks to be raised as layers here.

Figure 8: Trend in the percentage of MDR Salmonella isolated in local chicken layer farms between 2010 and Aug 2017

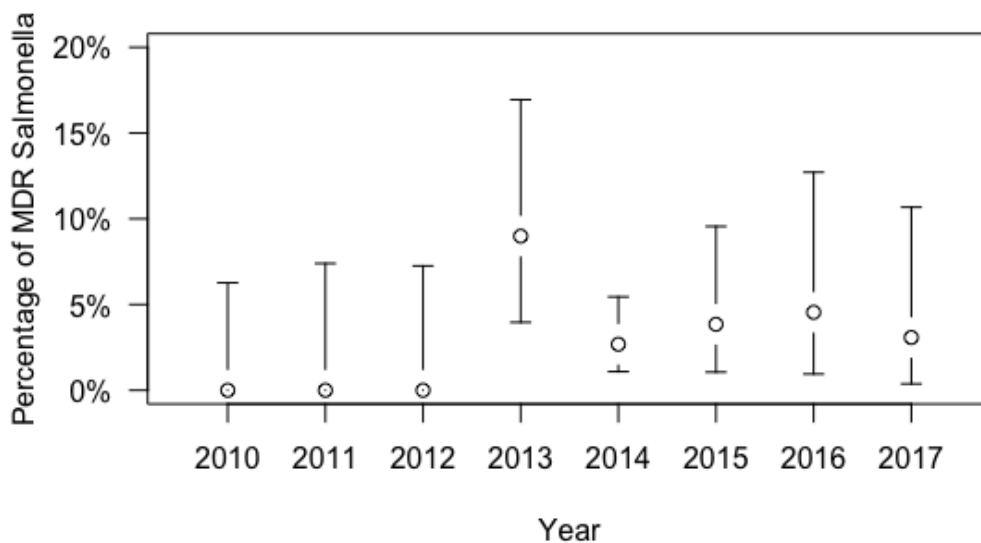
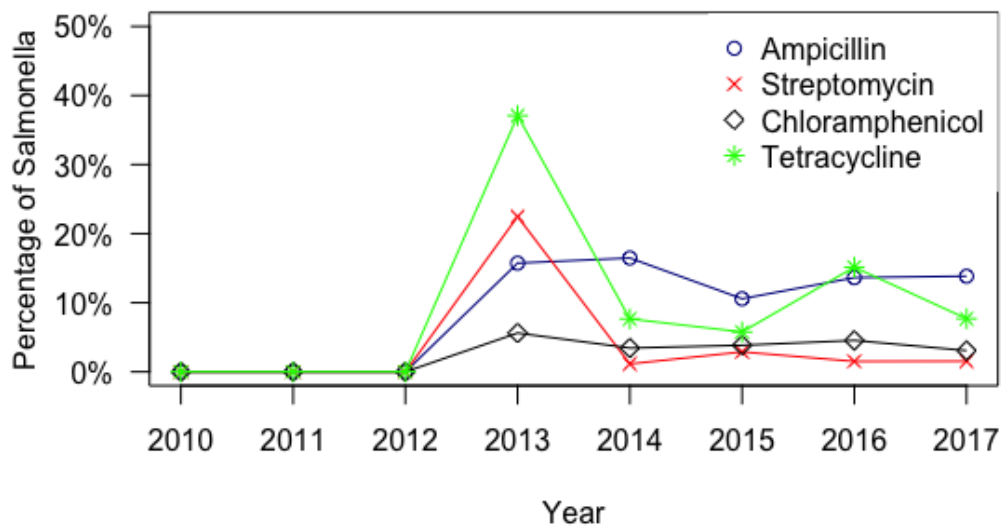


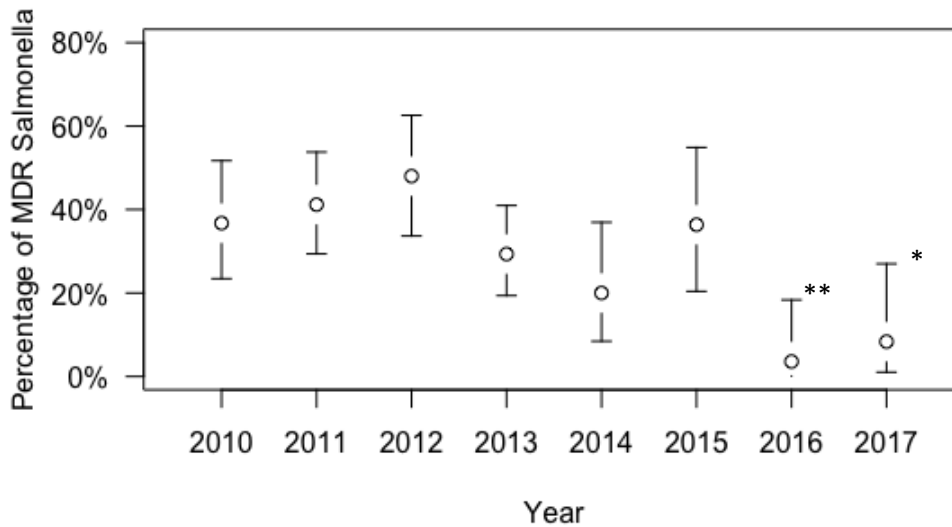
Figure 9: Trend in the percentage of Salmonella isolates from local chicken layer farm which are resistant to ampicillin, streptomycin, chloramphenicol and tetracycline from 2010 to Aug 2017.



RESISTANCE PROFILE OF SALMONELLA IN LOCAL QUAIL LAYER FARMS

From 2010 to 2017, 362 different *Salmonella* spp. were isolated from local quail farms. Of all isolates, 114 (31.5%) of *Salmonella* isolates were MDR. Overall, a significant decrease in MDR *Salmonella* was observed in 2016 (1 of 27; 4%) and 2017 (2 of 22; 9%) as compared to 2010 (37%) (Figure 10).

Figure 10: Trend in the percentage of MDR *Salmonella* isolated in local quail farms between 2010 and Aug 2017. * $p < 0.05$ and ** $p < 0.01$.



COLISTIN RESISTANCE GENE *MCR-1*

The first report of the *mcr-1* gene conferring plasmid-mediated colistin resistance in 2015⁷ was a cause for concern, as colistin is considered an antibiotic of last resort. Colistin was, at that time, widely used in agriculture in many parts of the world, though not in Singapore. A total of 394 *Salmonella* spp. isolated from 2014 to 2016 were screened for the presence of the *mcr-1* gene in isolates from local and imported animals. Of the isolates, 326 (83%) and 38 (10%) were isolated from local poultry farms and overseas poultry farms respectively. The *mcr-1* gene was detected in 2.8% (1/38) of *Salmonella* isolates from overseas poultry farms in 2016 and 0.3% of *Salmonella* isolates from local layer farms in 2014.

LA-MRSA IN IMPORTED PIGS

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has been reported in many species, but is mainly associated with pigs. To estimate the prevalence of LA-MRSA in pigs

⁷ Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J (2015). Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis.* 16:161-8.

imported for slaughter, nasal swabs were collected from 720 imported live pigs from Oct 2010 to Apr 2011. MRSA isolates were identified using a combination of molecular detection of the *mecA* gene, detection of penicillin binding protein via the latex agglutination test and antimicrobial susceptibility tests (AST). Multi-Locus Sequence Typing (MLST) was also used to characterise and type the isolates. A total of 276 MRSA isolates were recovered from the pigs, accounting for 38.3% of the pigs tested. Molecular typing showed that the isolates belonged to two Sequence Types: ST398 (10% or 28/276 isolates) and ST9 (90% or 248/276 isolates). This was consistent with reports showing that ST398 predominates in Europe and North America, whereas the majority of LA-MRSA isolates from Asia belong to ST9⁸.

MRSA AND MRSP IN COMPANION ANIMALS

A total of 137 (3.6%) methicillin-resistant Staphylococci were isolated from 3,801 companion animal samples from 2014 to 2017. Of the isolates submitted in 2017, 90% (45 of 50) were identified as methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), which is reportedly more commonly isolated from animals than humans.⁷ Further identification of the remaining isolates to species level is in progress.

⁸ Huber H, Koller S, Giezendanner N, Stephan R, Zweifel C. Prevalence and characteristics of methicillin-resistant *Staphylococcus aureus* in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland, 2009. Euro Surveill. 2010;15(16).

Antimicrobial Resistance in Imported Food Products

Singapore imports more than 90% of food from some 170 countries around the world. Surveillance of food imports is primarily for food safety purposes, and is based on performance-based tracking of the import sources and the levels of risk associated with the imported commodity. Sampling is also subject to risk categorisation, history of compliance, and the source and importers' track records.

The Veterinary Public Health Centre has maintained a monitoring programme for multi-drug resistant (MDR) *Salmonella* spp. in food products since 2008. All *Salmonella* isolates were serotyped by the Kaufmann-White classification scheme⁹ and tested for antibiotic susceptibility to six antibiotics (ampicillin, chloramphenicol, streptomycin, sulphonamides, ciprofloxacin and tetracycline) using disc diffusion methods. CLSI standards and human clinical breakpoints are applied (CLSI 100-S27E). AVA also monitors imported meat and poultry for the presence of MRSA, ESBL-producing *E. coli* and vancomycin-resistant Enterococcus (VRE).

When the *mcr-1* gene conferring colistin resistance was first reported by China in 2015, a study was conducted from 2015-2016 to assess the presence of the gene in current and archived *E. coli* and *Salmonella* isolated from food samples.

Findings from these programmes are summarised below. Owing to risk-based sampling and changing food sources, it should be noted that resistance data presented here should not be interpreted as the prevalence of resistant organisms in any specific source or food type.

RESISTANCE PROFILE OF SALMONELLA IN IMPORTED POULTRY

Salmonella spp. is naturally present in the digestive tracts in animals, and most frequently isolated from poultry and poultry products. Between 2008 to 2016, *Salmonella* was isolated from 4.6% (3,727 / 81,840) of all samples submitted for *Salmonella* testing, ranging from 2.3 to 6.7% per year. Raw chicken meat was the most common source of *Salmonella* spp., (41.3%, 1,538 / 3,727), followed by pork meat (11.3%, 421/3,727), duck meat (6%, 223/3,727) and other types of poultry such as quail and turkey (2.7%, 100/3,727).

Chicken for consumption in Singapore is largely imported either frozen or as live chickens from Malaysia for slaughter. Figure 11 shows the proportion of resistant *Salmonella* isolates from chilled (fresh) poultry carcasses sampled from local slaughterhouses from 2010 to June 2017, which are derived from live chickens imported from Malaysia. Figure 12 shows the proportion of resistant

⁹ Grimont P and Weill F. Antigenic formulae of the *Salmonella* serovars, 9th edition. WHO Collaborating Centre for Reference and Research on *Salmonella*, 2007.

Salmonella isolates from frozen poultry imported from South and North America over the same period.

All *Salmonella* isolates were tested for resistance to six antibiotics: ampicillin (AMP), chloramphenicol (C30), streptomycin (S10), sulphonamides (S3), ciprofloxacin (CIP) and tetracycline (TET). Two distinct patterns of resistance (Figures 11 and 12) could be observed in *Salmonella* spp. isolated from fresh and frozen poultry, which could point to different farming practices in the exporting countries.

Of isolates from chilled poultry, 54.3% (728/1339) were found to be MDR, compared with 82.3% (380/462) of isolates from frozen poultry from South America and 30.5% (101/331) of North American isolates. In contrast, chloramphenicol and tetracycline resistance were found to be relatively higher in chilled poultry isolates than frozen poultry isolates.

Although more than half of *Salmonella* isolates are multi-drug resistant, it should be noted that the overall detection of *Salmonella* in chicken is relatively low, with an average of 9.4% of chicken carcasses sampled at slaughterhouses, and 3.2% of frozen chicken meat sampled at import, over the past 5 years.

Figure 11: Proportion of *Salmonella* isolates from chilled poultry carcasses that are resistant to individual antibiotics ($n = 1339$)

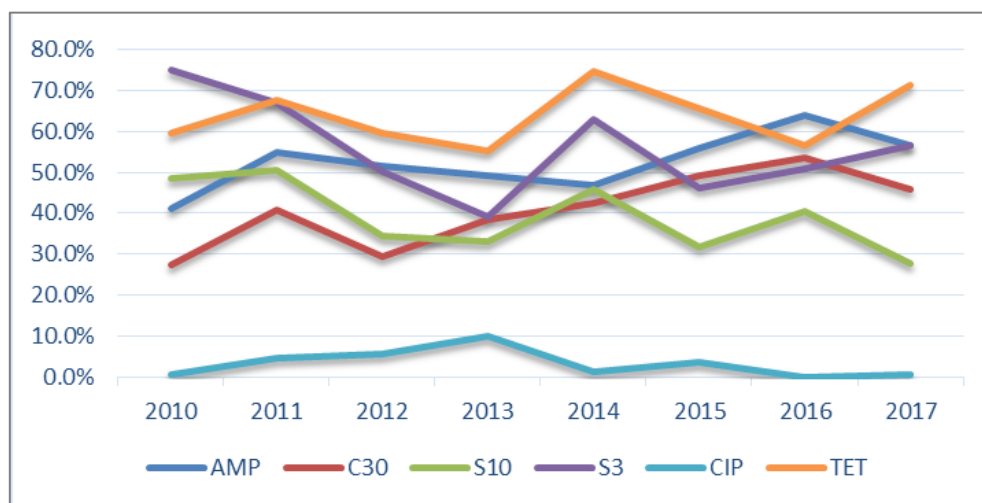
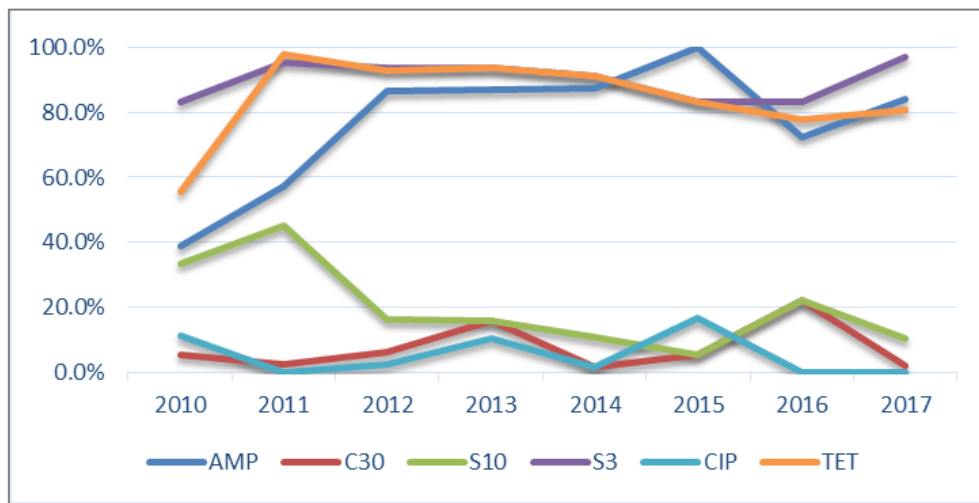


Figure 12: Proportion of *Salmonella* isolates from frozen poultry imported from North and South America that are resistant to individual antibiotics (n = 462)



MULTI-DRUG RESISTANCE AMONG COMMONLY ISOLATED SEROTYPES OF SALMONELLA

The average rate of *Salmonella* detection is approximately 4.6% of imported food product samples submitted for *Salmonella* testing from 2008 to 2016. The 10 most frequently isolated serovars identified in imported food products are shown in Figure 13. *Salmonella enterica* serovar Typhimurium and *S. enterica* ser. Enteritidis account for more than half of the serotypes isolated. However, MDR was most frequently detected among *S. enterica* ser. Albany and *S. enterica* ser. Brancaster, and less frequently among *S. enterica* ser. Enteritidis and *S. enterica* ser. Kentucky (Figure 14). Other frequently isolated serotypes possessed high levels (>50%) of MDR.

Figure 13: Ten most frequently isolated salmonella serovars from all imported food sources.

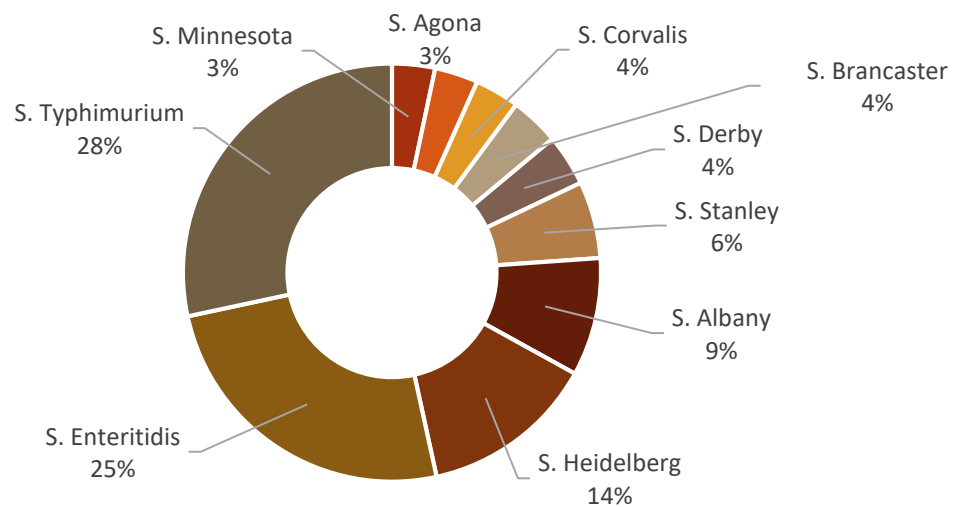
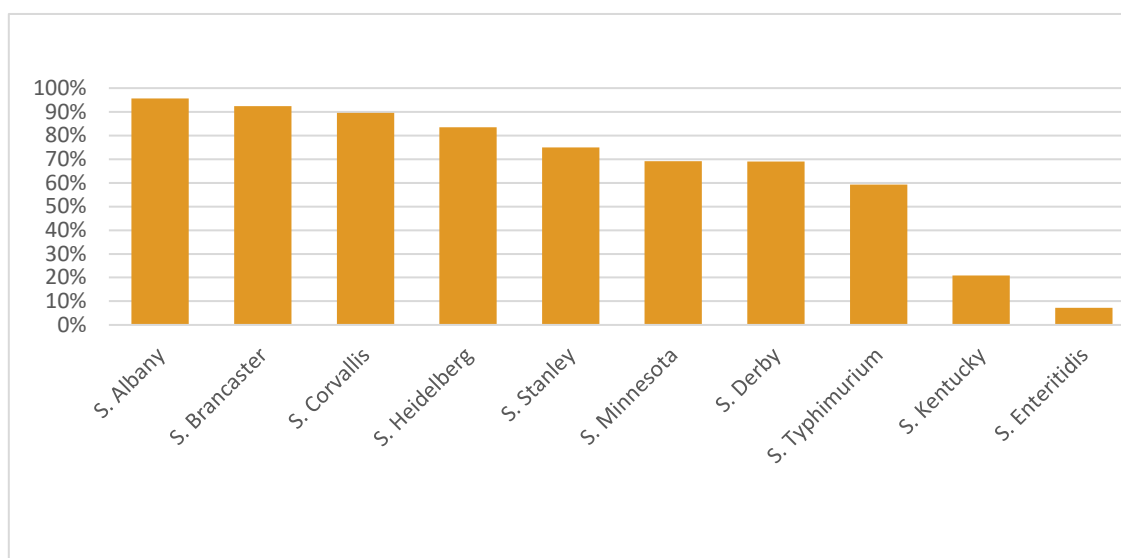


Figure 14: Proportion of MDR among the 10 most frequently isolated *Salmonella* serovars from 2010 to June 2017.



COLISTIN RESISTANCE GENE *MCR-1*

A study of 319 *Salmonella* spp. isolates from 2015 to 2016 and 601 *E. coli* isolates from 2014 to 2016 showed that the *mcr-1* gene was more frequently detected by PCR in *E. coli* isolates (18.14%) than in *Salmonella* isolates (1.25%). This suggested that there was thus far limited horizontal transfer of the *mcr-1* gene to *Salmonella* spp. However, 11% of *mcr-1* positive *E. coli* isolates in the study were resistant to both colistin and beta-lactam antibiotics. As the co-occurrence of *mcr-1* with other antibiotic resistance mechanisms is a cause for concern clinically, surveillance for *mcr-1* is now targeted at ESBL-producing *E. coli* isolates.

MRSA DETECTION FROM IMPORTED RAW MEAT

The rate of MRSA detection from imported raw meat (frozen or chilled poultry, pork, beef, mutton, lamb and game meats) from 2012 to 2017 was found to be very low, ranging from 0 to 0.52% (Table 3).

Table 3: Percentage of MRSA detections in frozen and chilled raw meat.

	2012	2013	2014	2015	2016	2017
Number of MRSA detected	5	1	0	0	5	1
Total Number of Samples Tested	963	940	1004	1060	1538	1551
Percentage of Resistance	0.52%	0.11%	0.00%	0.00%	0.33%	0.06%

VANCOMYCIN-RESISTANT ENTEROCOCCI DETECTION FROM IMPORTED CHILLED POULTRY

Detections of VRE in viscera of imported chilled poultry from 2012 to 2017 were mostly below 5% per year, with approximately 1000 samples tested each year (Table 4). There was a decrease in the number of chilled poultry tested in 2013 but a slight increase in the VRE detected in the same year (65/787). The majority of the VRE detections (>80%) for all years were identified as *Enterococcus durans*. The rest were other *Enterococcus* species such as *E. faecalis* and *E. gallinarum*.

Table 4: Percentage of VRE detections in imported chilled poultry

	2012	2013	2014	2015	2016	2017
Number of VRE detected	27	65	32	17	14	61
Total Number of Samples Tested	1010	787	1050	1125	1104	1097
Percentage Resistance	2.67%	8.26%	3.05%	1.51%	1.27%	5.56%
Number of <i>E. durans</i> Isolated	25	61	28	17	12	50
Percentage of <i>E. durans</i> Isolated	92.59%	93.85%	87.50%	100%	85.71%	81.87%

EXTENDED SPECTRUM BETA-LACTAMASE E. COLI DETECTION FROM IMPORTED AND LOCAL FOOD

A total of 1167 and 1090 *E. coli* isolates were tested in 2016 and 2017 respectively. Of these, 4.54% (53/1167) and 5.50% (60/1090) were respectively found to be ESBL-producing.

Antimicrobial Resistance in Retail Food Products

Retail food products monitored by the National Environment Agency (NEA) include cooked and ready-to-eat (RTE) food prepared and/or sold at retail food service premises (e.g. hawker centres, restaurants, coffee shops, caterers and food courts). Raw poultry, meat, vegetables and seafood products from wet markets and supermarkets are also monitored.

Through its retail food surveillance and research programme under the Environmental Health Institute (EHI), the NEA routinely tests for foodborne pathogens in retail food products. Bacteria isolated are also evaluated for antimicrobial susceptibility against clinically and epidemiologically important antimicrobial agents using disc diffusion.¹⁰ Bacteria genera/species tested include *Salmonella* spp., *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. Through this programme, the agency aims to ascertain the baseline occurrence and profiles of antimicrobial resistant pathogens in retail food products. The findings will ultimately provide evidence for shaping further surveillance, research and risk assessment studies on antimicrobial resistance in food, formulating policy and control measures, and evaluating the impact of the National Strategic Action Plan for AMR. Summary findings of the AMR surveys conducted by EHI between 2009 and 2016 are summarised below.

RESISTANCE PROFILE OF SALMONELLA

In a study to deepen our understanding of the extent of antimicrobial resistance in *Salmonella* from a One Health perspective, *Salmonella* isolates obtained from raw poultry (n=61), raw seafood (n=8), raw meat (n=7), human (n=152), wild birds and cooked/ready-to-eat food (n=18) samples were tested for their susceptibility to selected antimicrobials. The clinical isolates were obtained through a collaboration with KK Women's and Children's Hospital, Singapore General Hospital and Tan Tock Seng Hospital. Wild bird stool specimens were collected through EHI's zoonotic diseases surveillance programme.

We found *Salmonella* isolates resistant to at least one antimicrobial agent in human samples (38.2%, 58/152), cooked/RTE food (38.9%, 7/18) and raw food (26.3%, 20/76), but none in wild birds tested (0%, 0/15). Although the number of isolates from wild birds obtained over the past years is limited (n=15), our findings suggest a possibility of relatively lower antibiotic resistance selection pressure on *Salmonella* from the environment where these wild birds reside, than from the human and food sectors.

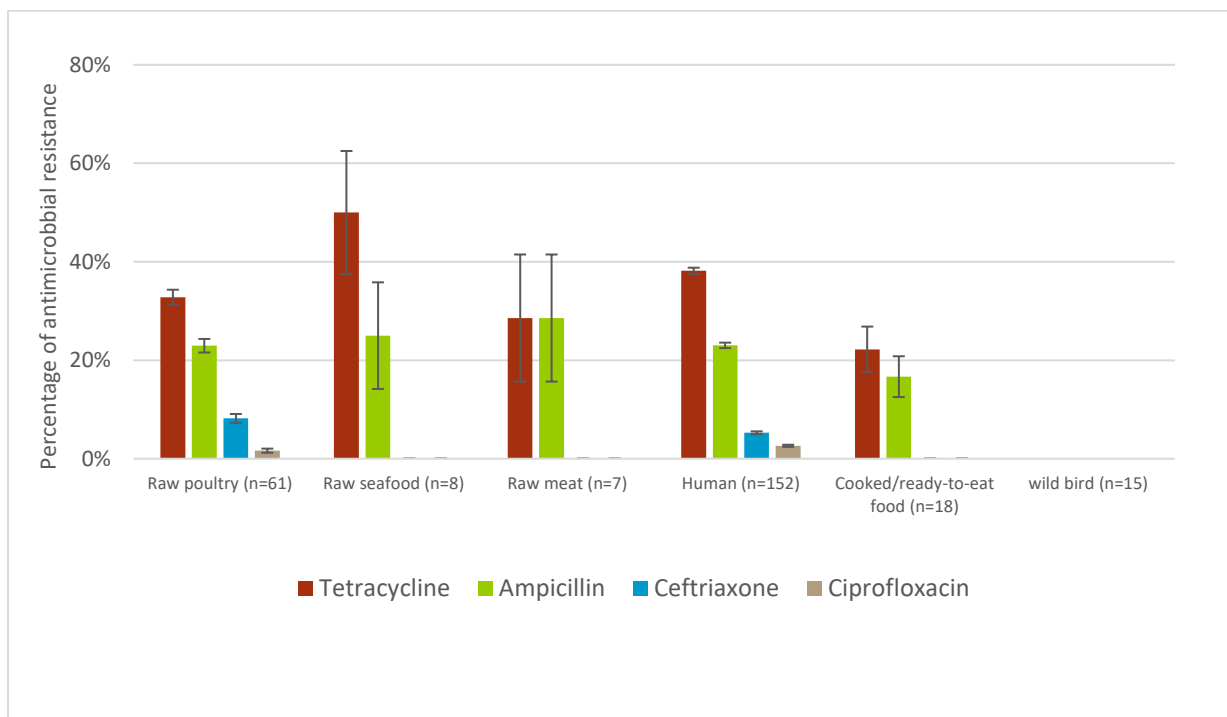
Tetracycline and ampicillin are commonly used antimicrobials for therapeutic or sub-therapeutic purposes in animal and agricultural sectors, and are considered drugs of choice for a number of human

¹⁰ CLSI, 2014. Performance standards for antimicrobial susceptibility testing; 24th informational supplement. CLSI document M100-S24.

infections.^{11, 12, 13} We found tetracycline and ampicillin resistant *Salmonella* isolates in raw poultry (32.8%, 20/61; 23.0%, 14/61), raw seafood (50.0%, 4/8; 25.0%, 2/8), raw meat (28.6%, 2/7; 28.6%, 2/7), human (38.2%, 58/152; 23.0%, 35/152) and cooked/RTE food (22.2%, 4/18; 16.7%, 3/18) respectively (Figure 15).

Resistance to two clinically important antimicrobials (ceftriaxone and ciprofloxacin) was found in *Salmonella* from raw poultry (8.2%, 5/61 and 1.6%, 1/61, respectively), and human samples (5.3%, 8/152 and 2.6%, 4/152, respectively). Ceftriaxone and ciprofloxacin are drugs of choice for treating systemic salmonellosis infections in humans.^{14, 15} The presence of *Salmonella* resistant to clinically important antimicrobials in raw poultry meat warrants further investigation, e.g. using genomic characterisation to elucidate antimicrobial resistance mechanisms, and to determine possible genetic relatedness of resistant isolates from raw poultry and human samples.

Figure 15: Percentage of antimicrobial susceptibility of *Salmonella* isolates from retail food products, human and wild birds



¹¹ Kumar K, Gupta SC, Chander Y and Singh AK. (2005) Antibiotic use in agriculture and its impact on the terrestrial environment. *Advances in Agronomy*. 87: doi:10.1016/S0065-2113(05)87001-4.

¹² Malik ZA and Litman N. (2006) Ampicillin and amoxicillin. *Pediatrics in Review*. 27(11).

¹³ Chopra I and Roberts M. (2001) Tetracycline antibiotics: mode of action, applications, molecular biology and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews*. 65(2): 232-260.

¹⁴ Wallace MR, Yousif AA, Mahroos GA, Mapes T, Threlfall EJ, Rowe B, and Hyams KC. (1993) Ciprofloxacin versus ceftriaxone in the treatment of multi resistant typhoid fever. *European Journal of Clinical Microbiology and Infectious Diseases*. 12(12): 907-910.

¹⁵ Anuforom O, Wallace GR, Buckner MMC, and Piddock LJV. (2016) Ciprofloxacin and ceftriaxone alter cytokine response, but not Toll-like receptors, to *Salmonella* infection in vitro. *Journal of Antimicrobial Chemotherapy*. 71(7): 1826-1833.

RESISTANCE PROFILE OF ENTEROBACTERIACEAE (*ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE*)

E. coli isolates were obtained from cooked and RTE poultry and fish-based dishes through routine food surveillance programme. Of 100 *E. coli* isolates, 17% (17/100), 15% (15/100), 10% (10/100) and 8% (8/100) were resistant to tetracycline, ampicillin, chloramphenicol and trimethoprim-sulphamethoxazole respectively. Lower rates of resistance towards nalidixic acid (4%, 4/100), ceftriaxone (2%, 2/100), ciprofloxacin (1%, 1/100), gentamicin (1%, 1/100), and norfloxacin (1%, 1/100) were found. No isolates in this study were resistant to amoxicillin-clavulanic acid, or amikacin (Figure 16). Twenty-four percent (24%, 24/100) of *E. coli* isolates were resistant to 3 or more classes of antimicrobials and thus were considered MDR strains. Two isolates (2% 2/100) were confirmed as ESBL- producing *E. coli* by double-disk synergy.¹⁶

A total of 97 *K. pneumoniae* isolates, obtained from raw, cooked and RTE food products through a cross-sectional research study in 2014, were subjected to antimicrobial susceptibility testing. The findings showed that, besides inherent resistance to ampicillin (97.9%, 95/97), the highest rates of antimicrobial resistance were against tetracycline (13.4%, 13/97), ciprofloxacin (8.3%, 8/97), and chloramphenicol (7.2%, 7/97). Lower rates of resistance were observed against amoxicillin-clavulanic acid (5.2%, 5/97), trimethoprim-sulphamethoxazole (5.2%, 5/97), nalidixic acid (2.1%, 2/97), amikacin (1.0%, 1/97), and ceftriaxone (1.0%, 1/97). No isolate in this study was resistant to norfloxacin or gentamicin (Figure 17). Five isolates (5.2%, 5/97) were resistant to 3 or more classes of antimicrobials and were considered MDR strains. The proportion of MDR in *K. pneumoniae* (5.2%, 5/97) was significantly lower ($p < 0.05$) than that of *E. coli* (24%, 24/100), suggesting that *E. coli* in food may be of higher competence than *K. pneumoniae* in taking up resistance traits upon exposure to the environmental selection pressure or from other resistant bacteria.

As these *E. coli* and *K. pneumoniae* isolates were obtained from retail cooked or RTE food dishes that were often mixed with garnishes, it was not possible to identify the origin of the antimicrobial resistant strains. Nevertheless, the presence of antimicrobial resistant and MDR non-foodborne-pathogenic *E. coli* and *K. pneumoniae* in RTE retail food suggests that poor food hygiene may be a silent pathway for the amplification and spread of AMR genes in the community. It reinforces a need for continuous and more encompassing education and constant reminders on food hygiene and safety, both to food handlers and consumers. Findings from this study provide phenotypic antimicrobial resistance rates and profiles of *E. coli* and *K. pneumoniae* found in local food that may be used for further in-depth investigations.

¹⁶ CDS, 2016. Antibiotic Susceptibility Testing by the CDS Method - Eighth Edition, Australia.

Figure 16: Percentage of antimicrobial susceptibility of *E. coli* isolates from retail food products

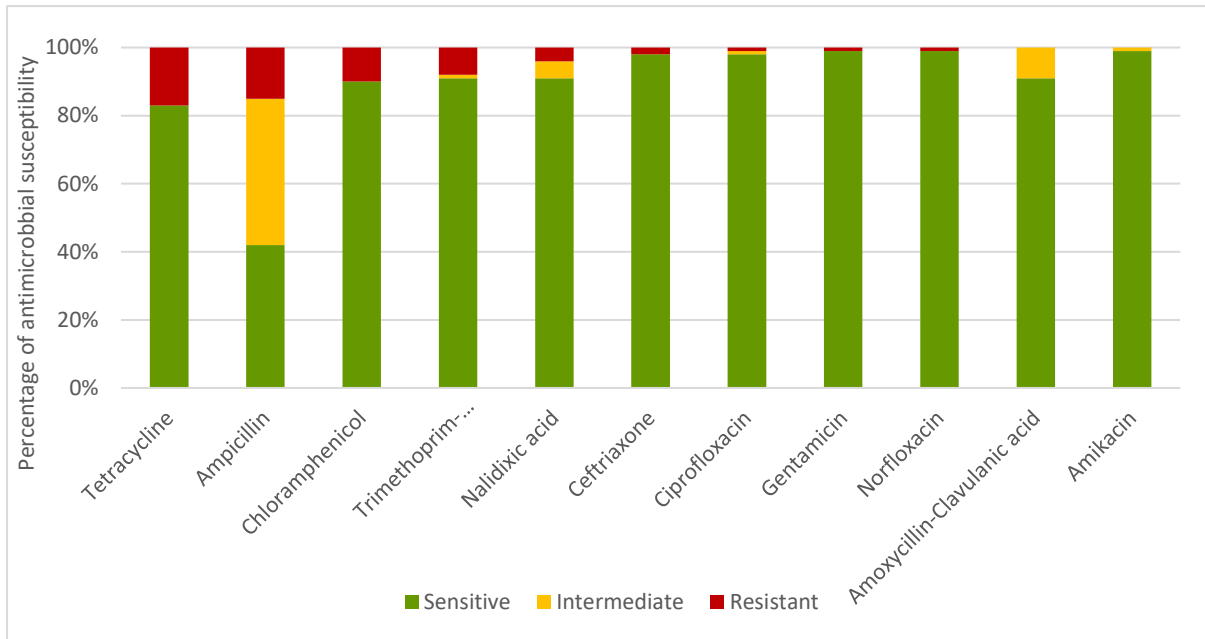
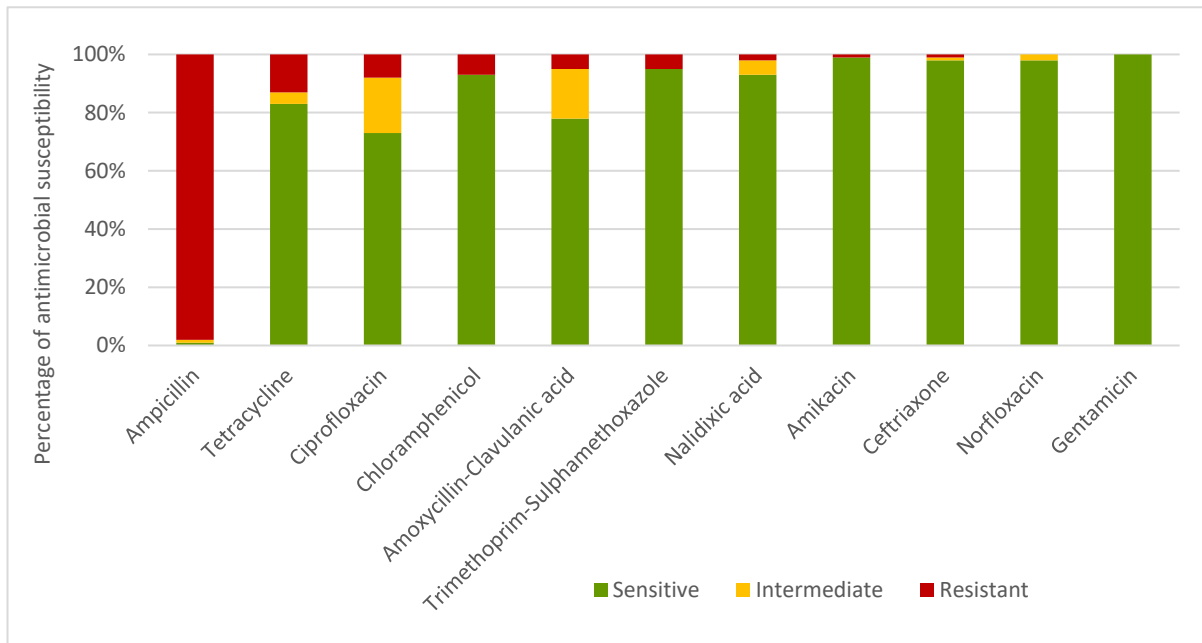


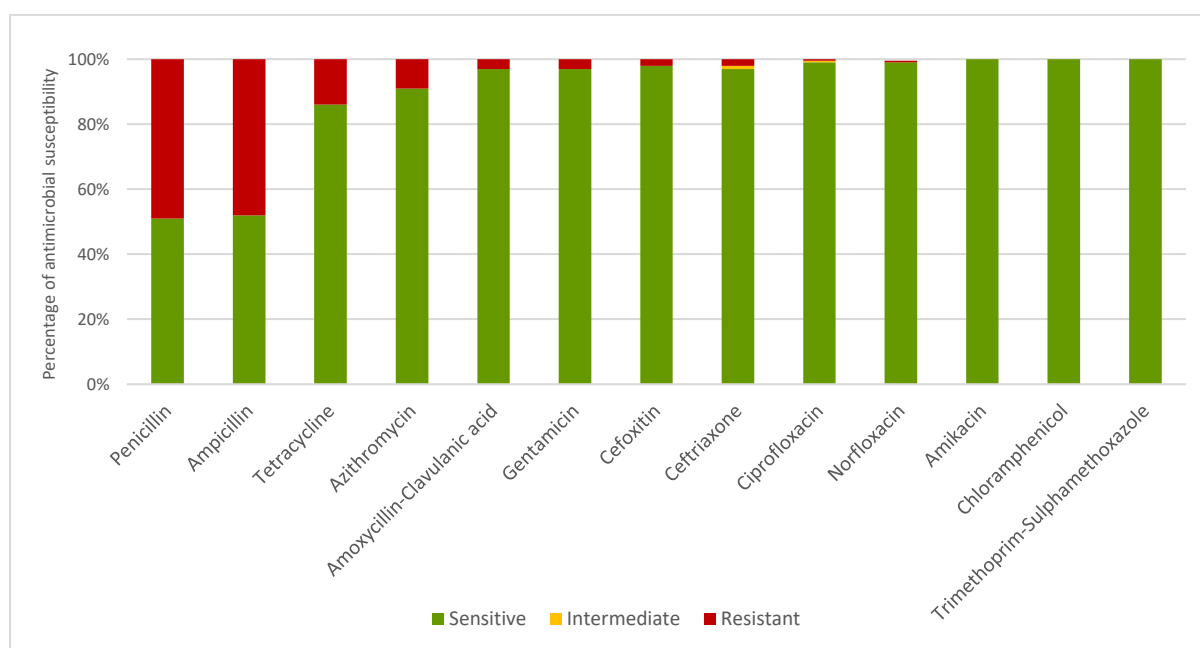
Figure 17: Percentage of antimicrobial susceptibility of *K. pneumoniae* isolates from retail food products



RESISTANCE PROFILE OF STAPHYLOCOCCUS AUREUS

Of 227 *S. aureus* isolates from cooked/RTE retail food products and food contact surfaces (e.g. gloves) obtained between 2011 and 2014, approximately 50% were resistant to penicillin (48.9%, 111/227) and ampicillin (48.5%, 110/227) (Figure 18). Resistance towards tetracycline and azithromycin were observed in 14.1% (32/227) and 8.8% (20/227) respectively (Figure 18). Approximately two percent (1.8%, 4/227) were MDR strains. Of 227 isolates, five (2.2%) were MRSA and belonged to sequence types ST80 and ST6. All five MRSA isolates belonged to staphylococcal cassette chromosome (SCC_{mec}) type IV, were Panton-Valentine leucocidin (*pvl*)-negative and staphylococcal enterotoxin genes (A or B)-positive.¹⁷ Based on the phenotypic and genotypic characteristics, MRSA isolates at retail were community associated (CA-MRSA) strains, more likely associated with human origins than with animals. Our findings thus far show no evidence of LA-MRSA (ST398 or ST9) in retail food products, which can be a potential point for entry and cross-sectoral transmission (a major public health concern) of MRSA from livestock to human (consumers). Nevertheless, our findings of clinically relevant MRSA sequence types in RTE food and food contact surfaces suggest food and food contact surfaces are potential environmental sources of MRSA colonisation and spread in the community.

Figure 18: Percentage of antimicrobial susceptibility of *Staphylococcus aureus* isolates from retail food products and food contact surfaces



¹⁷ Aung KT, Hsu LY, Koh TH, Chanditha HH, Chau ML, Gutiérrez RA, Ng LC. (2017) Enterotoxigenic panton-valentine leucocidin-negative sequence type (ST)80 and ST6 methicillin resistant *Staphylococcus aureus* isolated from food and food preparation environments in Singapore. *Antimicrobial Resistance and Infection Control*. 6: 94-97.

Antimicrobial Resistance in the Water Environment

Studies of hospitals and clinical environments in Singapore have shown rising antibiotic resistance levels since the mid-2000s^{18 19 20}. However, it is unclear as to what extent these antibiotic resistance levels have influenced the environment and in particular, the urban water cycle. Within the urban water loop, urban catchments and reservoirs serve as raw water sources for drinking water supply, while wastewaters are treated and discharged into the marine environment or reclaimed for indirect potable use. These different systems represent possible environments for the exchange of antibiotic resistance genes and spreading of antibiotic resistance.

Several analytical protocols have been developed in the studies discussed below, to examine levels of antibiotic resistant bacteria (ARB) and associated antibiotic resistant genes (ARG) in a variety of environmental waters. Samples were obtained from domestic and hospital wastewater, reservoirs and catchments. The AMR surveillance programme was conducted with phenotypic (traditional plate-count approach), and genotypic (PCR, qPCR, phylochip) detection methods for ARB and ARG. These approaches were used to compare the abundance of ARB and ARG between different sources of waters.

CONCENTRATION OF INDICATOR ORGANISMS IN WATER

A study was conducted to compare domestic wastewater and hospital wastewater, reservoirs and catchments. The findings showed that hospital wastewater had the highest occurrence of ARGs and ARBs (Figure 19).

The average loads of faecal indicators in the municipal and hospital wastewater influent were comparable (9.7×10^7 MPN/100ml and 1.7×10^7 MPN/100ml, respectively) and about three to six orders of magnitude higher than in the Water Reclamation Plants (WRP) (or Wastewater Treatment Plants) effluents after conventional treatment (1.3×10^4 MPN/100ml) and in the WRP effluents after the advanced Membrane Bioreactor (MBR) process (4 MPN/100ml).

Although the conventional treatment in the wastewater treatment process reduced the faecal indicator bacteria number with removal rates above 99%, a significant number of faecal bacteria remained in the wastewater effluent. The MBR process seemed to be much more efficient in

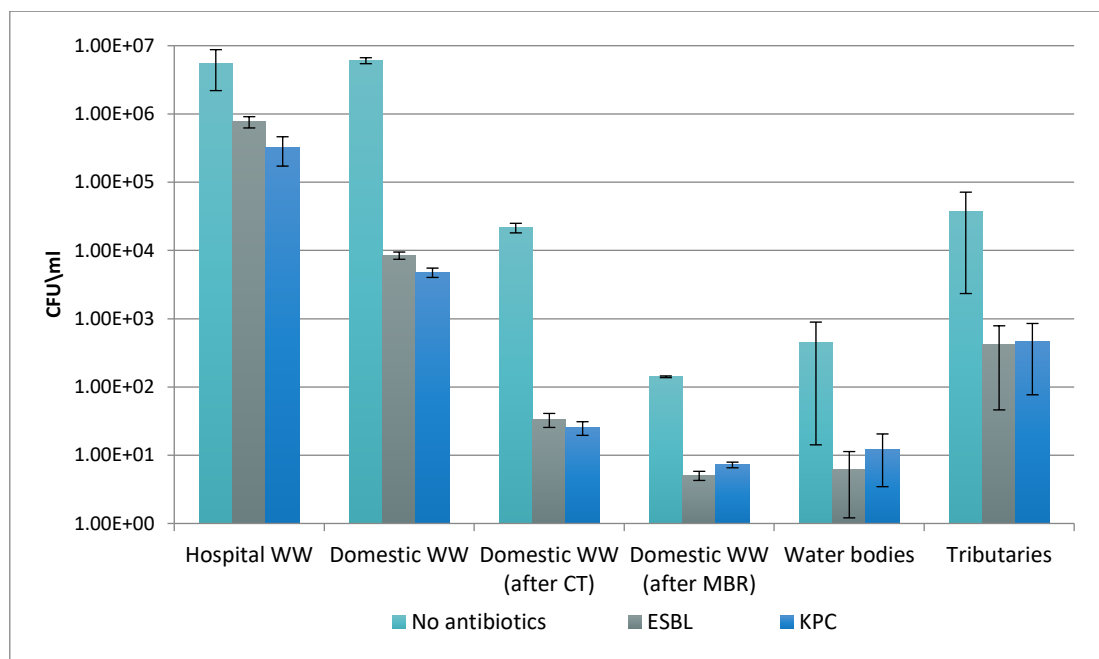
¹⁸ Hsu LY, Tan TH, Jureen R, Koh TH, Krishnan P, Lin RTP, Tee NWS and Tambyah PA (2007). Antimicrobial Drug Resistance in Singapore Hospitals. *Emerging Infectious Diseases*, 13: 1944 - 1947.

¹⁹ Liew YX, Krishnan P, Yeo CL, Tan TY, Lee SY, Lim WP, Lee W, Hsu LY and the Network for Antimicrobial Resistance Surveillance (Singapore). Surveillance of Broad-Spectrum Antibiotic Prescription in Singaporean Hospitals: A 5-Year Longitudinal Study. *PLoS ONE* 6:12.

²⁰ Hsu LY, Tan TY, Tam V, Kwa A, Fisher D A, Koh TH and the Network for Antimicrobial Resistance Surveillance (Singapore) (2010). Surveillance and Correlation of Antibiotic Prescription and Resistance of Gram-Negative Bacteria in Singaporean Hospitals. *Antimicrobial Agents and Chemotherapy*, 54(3):1173–1178.

eliminating almost all faecal organisms from the sewage completely. Counts in freshwater samples were relatively low with values below 100 MPN/100ml in reservoirs (water bodies used as a source of water supply) and between 2.5×10^3 and 4.3×10^3 MPN/100ml in catchments (tributaries, area from which rainfall flows into the reservoirs).

Figure 19: Gram-negative bacteria in water samples



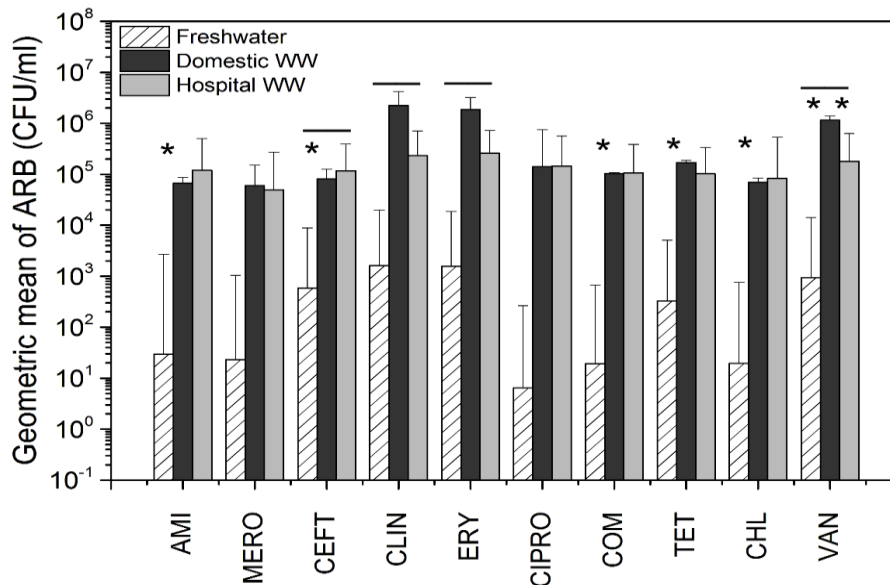
Note: Average concentrations of Gram-negative bacteria based on colony forming unit (CFU) counts per mL, on ESBL and KPC supplemented plates vs. non-supplemented plates, at various sampling points of the urban water cycle, including hospital wastewater (WW), domestic WW before treatment, domestic WW after conventional treatment (CT), domestic WW after the membrane bioreactor process (MBR) and freshwater samples. Error bars indicate standard deviation between 8 hospital wastewaters, 4 domestic wastewaters and 12 freshwater samples.

DISTRIBUTION OF ANTIMICROBIAL RESISTANT BACTERIA (ARB) CONCENTRATIONS IN WATER

The average concentrations of total ARB were two to three orders of magnitude higher in domestic (5.94×10^5 CFU/mL) and hospital wastewaters (1.40×10^5 CFU/mL) than freshwaters (5.14×10^2 CFU/mL). For each antibiotic, the differences in ARB concentrations between the three sample types indicated that the resistance levels in freshwaters were at least two magnitudes lower than domestic or hospital wastewaters (Figure 20). Bacterial resistance to clindamycin and erythromycin were the highest among the 10 antibiotics tested with concentrations in hospital and domestic wastewaters, and freshwaters in the order of 10^5 , 10^6 and 10^3 CFU/mL respectively (Figure 20). We found that the freshwaters had significantly lower concentrations of bacteria resistant to amikacin (2.97×10^1 CFU/mL), ceftazidime (5.83×10^2 CFU/mL), clindamycin (1.62×10^3 CFU/mL), erythromycin (1.57×10^3

CFU/mL), cotrimoxazole (1.92×10^1 CFU/mL), tetracycline (3.30×10^2 CFU/mL), chloramphenicol (1.95×10^1 CFU/mL) and vancomycin (9.36×10^2 CFU/mL), when compared to either of the wastewater samples (t-test, $P < 0.05$). The differences in resistance concentrations of domestic and hospital wastewaters were not significant, except for vancomycin resistance, where domestic wastewaters were one order of magnitude higher (1.15×10^6 CFU/mL).²¹

Figure 20. Concentrations of resistant bacteria in freshwater, domestic and hospital wastewaters



Note: Concentrations of bacteria resistant to the 10 antibiotics used for this study in freshwater (white shaded), domestic (black) and hospital (grey) wastewaters. Values are represented as geometric mean concentrations and error bars indicate standard deviation between 8 hospital wastewaters, 4 domestic wastewaters and 10 freshwater samples. The asterisk symbols indicate a significant difference ($P < 0.05$) between freshwater and domestic wastewaters, or domestic and hospital wastewaters, while the line indicates a significant difference ($P < 0.05$) between freshwaters and hospital wastewaters. Abbreviations of antibiotics are as follows; AMI – Amikacin, MERO – Meropenem, CEFT – Ceftazidime, CLIN – Clindamycin, ERY – Erythromycin, CIPRO – Ciprofloxacin, COM – Cotrimoxazole, TET – Tetracycline, CHL – Chloramphenicol, VAN – Vancomycin

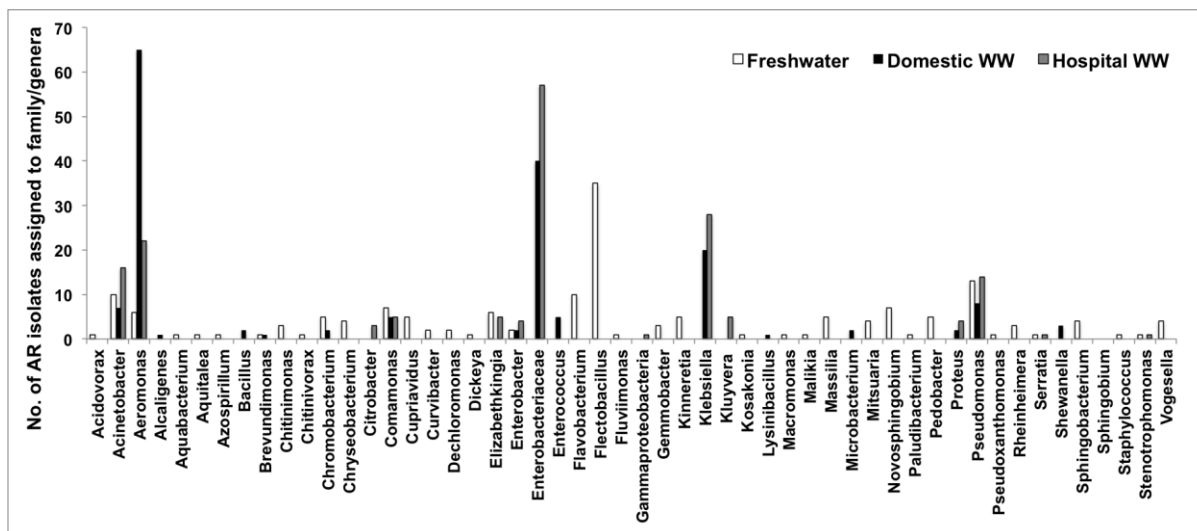
BACTERIAL COMPOSITION OF ANTIBIOTIC RESISTANT ISOLATES IN WATER SAMPLES

The 16S rRNA gene of 166 resistant isolates per water sample type was sequenced, which yielded a total of 498 partial 16S rRNA bacterial gene sequences. For the subset of 166 antibiotic resistant isolates picked from the freshwater samples, species belonging to *Flectobacillus* (20 %, 35 isolates), *Pseudomonas* (7 %, 13 isolates), *Acinetobacter* (6 %, 10 isolates), *Flavobacterium* (6 %, 10 isolates) and *Aeromonas* (6 %, 10 isolates) were a few of the most frequently detected AR genera (Figure 21). In contrast, *Aeromonas* spp., *Enterobacteriaceae* spp., *Klebsiella* spp., *Pseudomonas* spp. and

²¹ Low A, Ng C and He J (2016) Identification of antibiotic resistant bacteria community and a GeoChip based study of resistome in urban watersheds. *Water Research* 106: 330-338.

Acinetobacter spp. were the 5 most abundant taxa in the wastewaters. Species belonging to the Enterobacteriaceae family made up majority of the total number of isolates from hospital wastewaters (34%, 57 isolates), followed by *Aeromonas* (13 %, 22 isolates), *Acinetobacter* (10 %, 16 isolates) and *Pseudomonas* (8 %, 14 isolates). The domestic wastewaters had the same composition, with varying abundances, *Aeromonas* spp. (39 %, 64 isolates) being the most dominant, followed by species within the Enterobacteriaceae family (24 %, 40 isolates), *Klebsiella* spp. (12 %, 20 isolates), *Pseudomonas* spp. (5 %, 8 isolates), and *Acinetobacter* spp. (4 %, 7 isolates). We found a higher phylogenetic diversity of antibiotic resistant freshwater isolates (67 unique species) than domestic (27 unique species) and hospital wastewaters (28 unique species). Composite metagenomic sequence data also supported the same observed trends in total bacterial community diversity among the freshwater and wastewaters (data not shown).

Figure 21. Phylogenetic composition of antibiotic resistant isolates cultured from freshwater, domestic and hospital wastewaters



Note: Phylogenetic composition of 498 antibiotic resistant isolates cultured from freshwater (white), domestic (black) and hospital (grey) wastewaters. Data was normalized to 166 isolates per sample type.

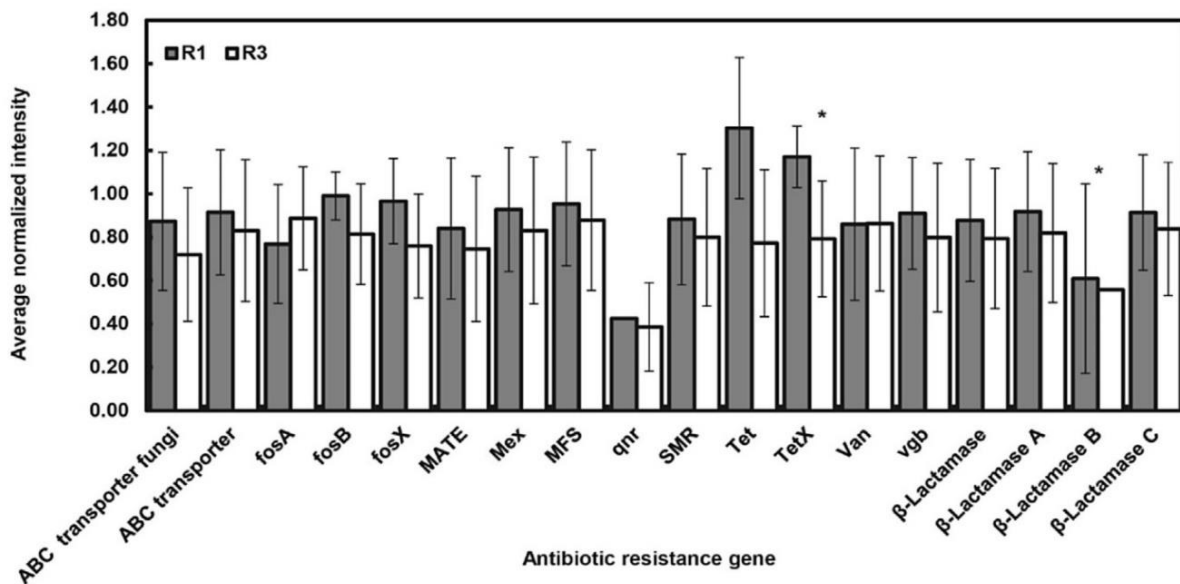
FREQUENCY OF DETECTION OF ANTIMICROBIAL RESISTANCE GENES (ARG) QUANTIFIED FROM DNA OF TWO WATERSHEDS

A watershed refers to an area of land that feeds all the water above and underground into a waterbody. A study²² was conducted on two watersheds in Singapore, R1 and R3, to investigate the diversity of ARGs using microarray. Using the GeoChip 5.0, which is equipped with probes that can detect various ARGs from different kinds of bacteria, 9,158 out of 15,850 probes corresponding to 18

²²Low A, Ng C and He J (2016) Identification of antibiotic resistant bacteria community and a GeoChip based study of resistome in urban watersheds. *Water Research* 106: 330-338.

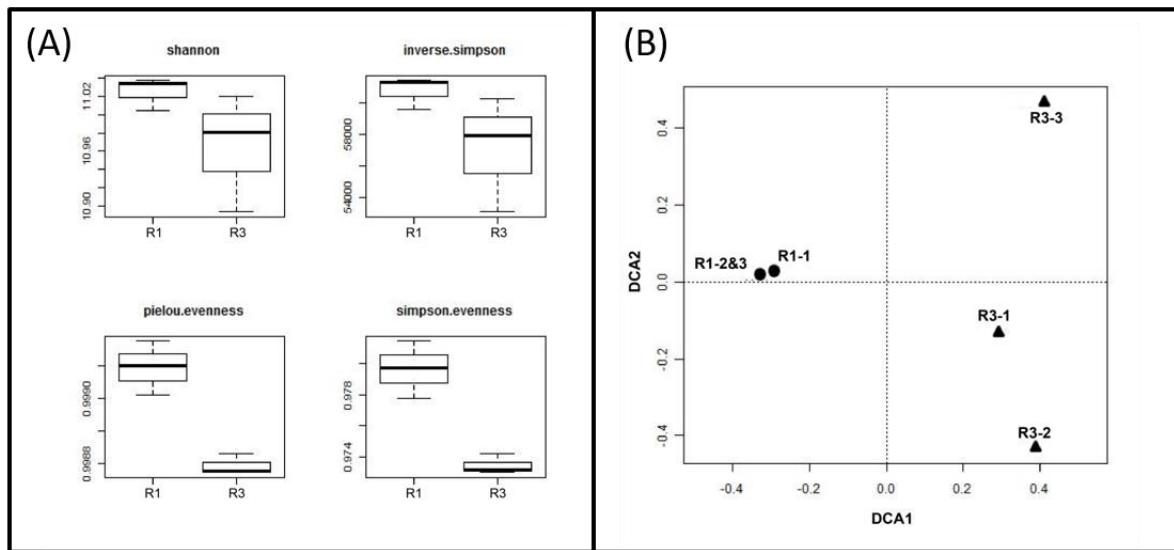
ARGs produced positive intensities. Comparison of the average normalised intensity showed that the R1 watershed had higher levels of genes corresponding to tetracycline and β -Lactam resistance (Figure 22). The results of the positive intensities corresponding to various bacteria were analysed for species richness or genetic diversity (Figure 23A, Shanon and Inverse Simpson) and species evenness (Pleulou and Simpson evenness). It was observed that R1 is more outstanding in terms of its ARB community diversity and evenness. A detrended correspondence analysis plot (Figure 23B of the gene and taxonomic compositions) also showed that the samples collected from R1 had higher levels of genetic similarity. Comparison of the level of antibiotic resistance (based on HPC with R2A agar) on the watersheds by Analysis of Similarity (ANOSIM) revealed that the R1 watershed had statistically more bacteria resistant to six of the seven antibiotics tested compared to the R3 watershed (Figure 24). R1, located in a more urbanised landscape, likely receives runoff that has higher nutrient content than R3. The R3 watershed receives water from a mixture of forested and less urbanised landscapes as well as from other watersheds, hence, the nutrient levels in these waters are expected to be lower because of dilution and shorter retention time in source watersheds.

Figure 22. Histogram of normalised total intensities of antibiotics resistance genes from two watersheds in Singapore (R1 & R3)



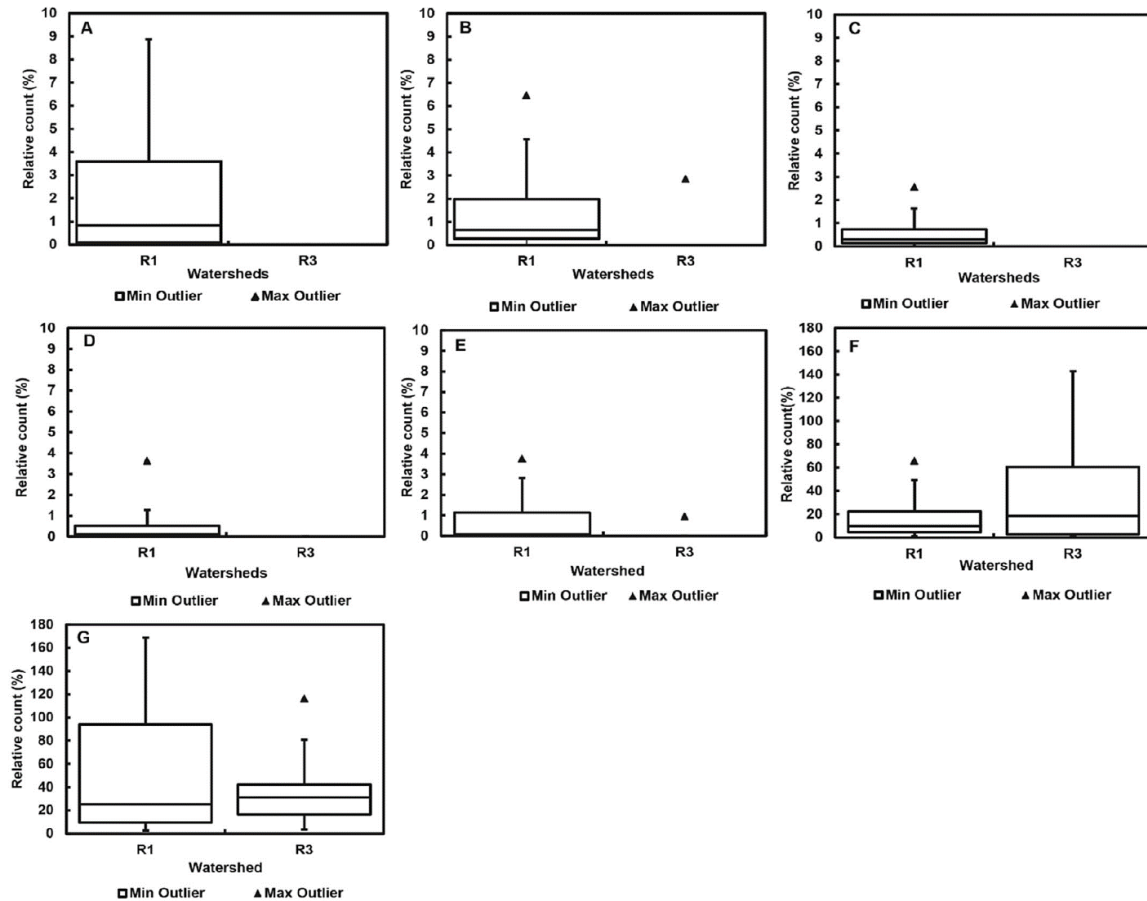
Note: Error bars show standard deviation of triplicate samples. Asterisk indicates significant difference of $p < 0.05$ (unpaired t-test).

Figure 23. Comparison of bacterial genetic diversity and species evenness in watersheds R1 and R3



Note (A) Boxplot comparisons of genetic diversity based on Shannon and Inverse Simpson indices and evenness based on Pielou and Simpson evenness indices. (B) A Detrended correspondence analysis (DCA) plot of R1 and R3 based on GeoChip analyses. R1-2 and 3 symbols overlap. Numbers following sample names indicate sampling locations 1, 2 or 3.

Figure 24. Relative counts of bacteria resistant to erythromycin (A), kanamycin (B), norfloxacin (C), sulfanilamide (D), tetracycline (E), lincomycin (F) and trimethoprim (G) in watersheds R1 and R3



Conclusion and Next Steps

As with AMU data, antimicrobial resistance data is comprehensive and closely-monitored in Singapore public hospitals, and covers all major pathogens of concern. Trends in resistance are closely monitored and regularly reported through NARCC, to MOH and hospitals for their action. Surveillance shows that the rates of ciprofloxacin and ceftriaxone resistance in *E. coli* and *K. pneumoniae* are higher than the rates of other pathogen-antibiotic combinations under surveillance. The organism with the highest incidence density per 10,000 inpatient days is *E. coli*, consistent with the overall trend of increasing resistance in Enterobacteriaceae. The incidence of *C. difficile*, which is a marker of total antibiotic use burden, has reduced, a reflection of hospitals' antimicrobial stewardship and infection prevention efforts. An increase in screening isolates for CPE also reflects active surveillance by hospitals to identify carriers early so that enhanced infection control measures can be undertaken to reduce such cases.

AMR surveillance in hospitals complements antimicrobial stewardship programmes and infection prevention and control measures, to reduce inappropriate use and reduce spread of infection within hospitals. At the time of this report, surveillance for drug resistant organisms is currently limited to the public hospitals. Work is underway to include private hospitals as part of national surveillance and explore submission of national AMR data to the Global Antimicrobial Resistance Surveillance System (GLASS) to support global data collection and for benchmarking purposes.

AMR bacteria in the food chain is a concern and efforts are being made to expand surveillance in this area. *Salmonella* has been the focus of national surveillance efforts, being a major cause of foodborne-associated illness both in Singapore and globally. The average annual prevalence of MDR *Salmonella* in local poultry farms is low (3.2% in chicken farms, 8% in quail farms in 2017), which correlates with the low sales of antimicrobials to the poultry sector. In comparison, MDR *Salmonella* in imported poultry and poultry products ranged from 54.3% to 82.3%. However, these higher MDR rates should be taken in context of the relatively low rates of *Salmonella* contamination, which is approximately 3.2% in frozen poultry at point of import and 9.4% in freshly slaughtered carcasses. Furthermore, import controls are placed on consignments and farms contaminated with *Salmonella*, particularly with *Salmonella enterica* ser. Enteritidis, which is the leading cause for non-typhoidal salmonellosis in Singapore. *Salmonella* is seldom isolated from cooked/RTE food, and infrequent detections tend to be associated with food containing poultry products. Strict regulations along the entire food chain have kept contamination rates low, but food pathogens, including drug-resistant food pathogens, are detected from time-to-time. These results highlight the importance of reinforcing safe food practices and good personal hygiene to reduce exposure to pathogens and drug-resistant organisms in food. Efforts are currently being made to expand surveillance to local livestock aquaculture farms, as well as to monitor resistance in indicator organisms, such as *E.coli*.

AMR bacteria from hospital, domestic and agriculture waste can contaminate the environment, if not adequately treated before release. Proper sanitation and effective waste treatment system are therefore essential in reducing levels of contamination to the environment. The presence of bacteria

in local watersheds is mitigated by effective water treatment systems in place in Singapore: studies on local wastewater treatment systems have found that the advanced Membrane Bioreactor treatment process is effective in removing contaminating bacteria from wastewater. Nevertheless, data from environmental surveillance of AMR bacteria and genes will be used to assess the risk of AMR in urban waters.

This first report compiles independent surveillance activities in the human, animal, food and environment sectors. Moving forward, the objective will be to build a more integrated national surveillance system that can provide information on priority pathogens across all sectors and apply the data to risk assessment and control measures for AMR.

List of Abbreviations

AHL	Animal Health Laboratory
AMR	Antimicrobial resistance
AMRCO	Antimicrobial Resistance Coordinating Office
AMU	Antimicrobial utilisation
ANOSIM	Analysis of Similarity
ARB	Antibiotic resistant bacteria
ARG	Antibiotic resistant genes
ASP	Antimicrobial Stewardship Programmes
AST	Antibiotic susceptibility testing
AVA	Agri-Food and Veterinary Authority
CA-MRSA	Community-associated methicillin-resistant <i>Staphylococcus aureus</i>
CDSS	Computerised decision support systems
CLSI	Clinical & Laboratory Standards Institute
CPE	Carbapenemase-producing Enterobacteriaceae
DDD	Defined daily doses
EHI	Environmental Health Institute
ESBL	Extended spectrum beta-lactamase
LA-MRSA	Livestock-associated methicillin-resistant <i>Staphylococcus aureus</i>
MBR	Membrane Bioreactor Process
MDR	Multi-drug resistant
MLST	Multi-Locus Sequence Typing
MOH	Ministry of Health
MPN	Most Probable Number
MRSA	Methicillin resistant <i>Staphylococcus aureus</i>
MRSP	Methicillin resistant <i>Staphylococcus pseudointermedius</i>
NARCC	National Antimicrobial Resistance Control Committee
NAREP	National Antimicrobial Resistance Expert Panel
NASEP	National Antimicrobial Stewardship Expert Panel
NAT	National Antimicrobial Taskforce
NCID	National Centre for Infectious Diseases
NEA	National Environment Agency
OIE	World Organisation for Animal Health
PCR	Polymerase Chain Reaction
qPCR	Quantitative PCR
SE	<i>Salmonella</i> Enteritidis
STM	<i>Salmonella</i> Typhimurium
VPHC	Veterinary Public Health Centre
VRE	Vancomycin-resistant Enterococci
WHO	World Health Organization
WRP	Water Reclamation Plant

Glossary

Aquaculture	Aquaculture refers to the farming of fish, crustaceans, molluscs and other organisms in water environments.
Antimicrobial agents	Antimicrobial agents include antibacterial, antiviral, coccidiostatic and antimycotic agents.
Broiler	A chicken raised for meat production.
Catchments	Tributaries, area from which rainfall flows into the reservoirs.
Clinical samples	Clinical samples are samples obtained as part of the management of a patient's medical condition.
Companion animals	Companion animals refers to pets such as dogs, cats, birds, rabbits and other pocket pets such as hamsters.
Defined daily dose (DDD)	DDD is the average daily maintenance dose for its main indicated use in adults and is a standard determined by the World Health Organization (WHO).
ESBL	Extended spectrum beta-lactamases (ESBL) are enzymes that cause resistance to extended spectrum (third generation) cephalosporins and monobactams, but do not affect cephamycins or carbapenams.
Food fish	Food fish refers to fish that are reared for the purpose of human consumption, as opposed to for ornamental purposes.
Layer	A hen raised to produce eggs for human consumption.
Multi-drug Resistance (MDR)	Resistance to at least three classes of antimicrobials.
Poultry	Poultry includes broilers, layers, duck, turkey, quail and geese.
Reservoir	Water bodies used as a source of water supply.
Screening samples	Screening samples are samples obtained from asymptomatic individuals for the purpose of surveillance and/or infection control.
Watershed	An area of land that feeds all the water above and underground into a waterbody.