



One Health Report on **Antimicrobial Utilisation and Resistance, 2021**

One Health Antimicrobial Resistance Workgroup,
Singapore

One Health Report on Antimicrobial Utilisation and Resistance in Singapore, 2021

A Report by the One Health Antimicrobial Resistance Workgroup

Participating ministries and agencies:



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This Report on Antimicrobial Utilisation and Resistance in Singapore is published by the One Health Antimicrobial Resistance Workgroup, comprising representatives of the Ministry of Health (MOH), Health Promotion Board (HPB), National Environment Agency (NEA), National Parks Board (NParks), PUB, the National Water Agency, and Singapore Food Agency (SFA). This report was compiled with the assistance of the Antimicrobial Resistance Coordinating Office (AMRCO), National Centre for Infectious Diseases (NCID).

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- Tan Tock Seng Hospital
- Singapore General Hospital
- Sengkang General Hospital
- Gleneagles Hospital
- Mount Elizabeth Hospital
- Mount Elizabeth Hospital (Novena)
- Mount Alvernia Hospital
- Parkway East Hospital
- Raffles Hospital
- Thomson Medical Centre
- Farrer Park Hospital
- National Healthcare Group (NHG) polyclinics
- National University Health System (NUHS) polyclinics
- Singapore Health Services (SingHealth) polyclinics

List of Abbreviations

AMR	Antimicrobial Resistance
AMRCO	Antimicrobial Resistance Coordinating Office
AMRWG	Antimicrobial Resistance Workgroup
AMU	Antimicrobial utilisation
ANIMUSE	WOAH's Global Database on ANImal antiMicrobial USE (ANIMUSE)
ARB	Antibiotic resistant bacteria
ARG	Antibiotic resistant genes
ASP	Antimicrobial stewardship programme(s)
AST	Antibiotic susceptibility test/testing
ATC	Anatomical Therapeutic Chemical
AVS	Animal & Veterinary Service, NParks
CAVS	Centre for Animal & Veterinary Sciences, NParks
CFU	Colony-forming unit
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CP-CRE	Carbapenemase-producing carbapenem-resistant Enterobacterales
CPE	Carbapenemase-producing Enterobacterales
CR	Carbapenem-resistant
CRE	Carbapenem-resistant Enterobacterales
CRO	Ceftriaxone
CTA	Countries, Territories & Areas
CTX	Cefotaxime
DDD	Defined Daily Dose
DID	DDD/ 1000 inhabitants/ day
EHI	Environment Health Institute, NEA
EPA	United States Environmental Protection Agency
ESBL	Extended spectrum beta-lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FIB	Faecal indicator bacteria
GI	Gastrointestinal
GLASS	Global Antimicrobial Resistance Surveillance System
GP	General practitioner
HIC	High Income Countries
HIV	Human Immunodeficiency Virus
HPB	Health Promotion Board
ID	Infectious Disease
IPM	Imipenem
IQR	Interquartile range
MDR	Multi-drug resistant
MEM	Meropenem
MIC	Minimum Inhibitory Concentration
MMP	Market Monitoring Programme
MOH	Ministry of Health
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>

MRSP	Methicillin-resistant <i>Staphylococcus pseudintermedius</i>
NAFTEC	Nanyang Technological University Food Technology Centre
NARCC	National Antimicrobial Resistance Control Committee
NCFS	National Centre for Food Science, SFA
NCID	National Centre for Infectious Diseases
NEA	National Environment Agency
NParks	National Parks Board
NPHL	National Public Health Laboratory
NTU	National Technological University
NUS	National University of Singapore
WOAH	World Organisation for Animal Health
PUB	Public Utilities Board, Singapore's National Water Agency
QMRA	Quantitative microbial risk assessment
SDG	Sustainable Development Goals
SFA	Singapore Food Agency
TNRM	Trap-Neuter-Release/Rehome-Manage
VRE	Vancomycin-resistant Enterococci
WGS	Whole Genome Sequencing
WHO	World Health Organization

Executive Summary

Antimicrobial resistance (AMR) is a natural phenomenon that occurs when microorganisms develop or acquire resistance to antimicrobials, rendering them ineffective. These antimicrobial resistant microorganisms can be found in humans, animals, and the environment (both natural and man-made), and their development is accelerated by the overuse and misuse of antimicrobials in humans, animals and agriculture. Surveillance serves to monitor the levels of AMR in the affected sectors and to track the consumption of antimicrobials by humans and animals. This provides important data and evidence needed to assess risks, guide policy decisions and measure the impact of interventions. Surveillance therefore forms a core strategy of Singapore's National Strategic Action Plan to combat AMR.

This One Health report updates on key findings from national AMR and antimicrobial utilisation (AMU) surveillance programmes up to the end of 2021. The last report can be accessed at <https://www.moh.gov.sg/resources-statistics/reports/one-health-report-on-antimicrobial-utilisation-and-resistance-2019>. This edition also contains the following new areas of surveillance:

- AMR and AMU data from private acute care hospitals
- AMR and AMU data from polyclinics
- Extended spectrum beta-lactamase producing (ESBL) *E. coli* (ESBL-Ec) in imported and retail food products
- AMR in free-roaming dogs
- AMR in wildlife
- ESBL-Ec in the environment

With the further expansion of AMR surveillance programmes, data from target populations and surveillance sites will contribute towards the formulation of science- and risk-based targets to further drive AMR control efforts.

HUMAN HEALTH

Utilisation of antimicrobials

In acute care hospitals (public and private), beta-lactams and beta-lactamase inhibitors were the most utilised group of antimicrobials, at 665.01 Defined Daily Doses (DDD) per 1000 inpatient days, followed by fluoroquinolones at 202.2 DDD per 1000 inpatient days. Overall, the utilisation of antimicrobials by acute care hospitals declined from 2019 to 2021, the period coinciding with the COVID-19 pandemic. The reduction of fluoroquinolone utilisation in 2021 was driven largely by a reduction in oral ciprofloxacin use. Carbapenem utilisation by acute care hospitals was also on a declining trend from 2017 to 2021. Different patterns of antimicrobial utilisation were observed between public and private acute care hospitals.

In public primary care settings, data collected from polyclinics showed an overall decline in the utilisation of oral antimicrobials since 2018. In 2021, beta-lactams and beta-lactamase inhibitors (15.0 DDD per 100 doctor visits) were the most utilised antimicrobial group, followed by tetracyclines (7.9 DDD per 100 doctor visits) and macrolides and lincosamides (2.2 DDD per 100 doctor visits).

Estimates of national consumption of antimicrobials currently rely on sales data obtained from private sector market research. In 2021, the sales of beta-lactams and penicillins (mainly amoxicillin-beta-lactamase inhibitors) were the highest, followed by tetracyclines (mainly doxycycline) and fluoroquinolones (mainly ciprofloxacin). Sales to private outpatient clinics were higher than sales to polyclinics, pharmacy, hospitals. We observed a substantial reduction in total reported sales volume of J01 antibiotics in 2020 and 2021, falling from 11.6 DDD per 1000 inhabitants/day (DID) in 2019 to 6.6 DID to 2021, due to reduced sales to private sector outpatient clinics.

Antimicrobial resistance rates

In acute care settings, the overall incidence of carbapenem-resistant *A. baumannii* has declined, while the incidences of carbapenem-resistant *E. coli*, *K. pneumoniae* and *P. aeruginosa* remained relatively stable in 2021. However, the incidence of ciprofloxacin-resistant *E. coli* and *K. pneumoniae* has been on an increasing trend in both public and private acute care hospitals since 2017 despite a declining use of fluoroquinolones. Carbapenemase-producing Enterobacterales are a growing concern worldwide and continue to be closely monitored. The OXA-type beta-lactamase was the most frequently detected carbapenemase across all acute care hospitals.

In primary care (polyclinic) settings, resistance proportions (%R) of priority community pathogens were generally stable from 2019 to 2021. In 2021, *E. coli* displayed the highest resistance to ampicillin (49.2%), ciprofloxacin (33.1%) and co-trimoxazole (25.8%). *K. pneumoniae* displayed the highest resistance to nitrofurantoin (81.1%) and ciprofloxacin (20.3%), while 4.8% of *S. aureus* isolates were MRSA. The overall number of carbapenemase producers remains very low.

At the international level, Singapore enrolled in the WHO Global AMR Surveillance System (GLASS) in 2019 to support global data collection and benchmarking. Based on data from Singapore's GLASS sentinel surveillance sites, the proportion of bloodstream *E. coli* resistant to 3rd-generation cephalosporins reported in 2021 was 25.8%, which was higher than the median of 13.3% (IQR 8.9 – 26.8%) of 35 high income countries (HICs) reporting to GLASS. Likewise, the proportion of bloodstream *S. aureus* resistant to methicillin in 2021 was 32.5%, higher than the median of 11.0% (IQR 4.3 – 33.1%) of 32 HICs reporting to GLASS.

ANIMAL HEALTH

In 2021, NParks expanded its survey base to include additional companies supplying veterinary antimicrobials to the animal sector. Of these, 73% of wholesalers provided data on antimicrobial sales, which contributed to the increased total quantity (in kg) reported in 2021 compared to 2019. Sales were largely to the aquaculture sector. In contrast, sales in the companion animal sector have generally remained stable despite a growing companion animal population. Tetracyclines, fluoroquinolones and penicillins were the most used antimicrobials in the veterinary sector from 2015 to 2021.

Passive AMR surveillance of *E. coli* from sick companion animals in 2020 to 2021 found that isolates were mostly resistant to ampicillin but susceptible to colistin and meropenem. Isolates also demonstrated resistance to medically important antimicrobials such as ciprofloxacin (23.1% in 2021) and third generation cephalosporins such as ceftazidime (15.4%) and cefotaxime (23.1%). *E. coli* and *K. pneumoniae* isolated from healthy free-roaming dogs in 2020-2021 exhibited similarly high

prevalence of resistance to ampicillin and susceptibility to colistin and meropenem. However, resistance rates of *E. coli* from free-roaming dogs were generally lower than those isolated from sick companion animals. MRSA was not isolated from either sick companion animals or healthy free-roaming dogs tested in 2020 and 2021. In 2020, NParks also initiated AMR surveillance of local wildlife such as palm civets, wild boars, macaques and bats. Wildlife *E. coli* isolates exhibited relatively low prevalence of resistance to tetracycline (8.5%), sulfamethoxazole (5.1%) and ampicillin (3.4%) while all were susceptible to ceftazidime, ciprofloxacin, gentamicin and meropenem. *E. coli* isolated from wildlife revealed distinct AMR patterns and lower prevalence of resistance than *E. coli* isolated from food-producing and companion animals, suggesting that exposure to anthropogenic activity could contribute to increased AMR.

FOOD PRODUCTION - LIVESTOCK

The overall prevalence of *Salmonella* spp. in local poultry farms ranged from 3.4% to 14.6% in 2020. In terms of resistance levels, the proportion of MDR *Salmonella* spp. was higher in quail farms (22.6%) than in chicken farms (1.2%) with none of the isolates being serovar Enteritidis. MDR *S. Enteritidis* and *S. Typhimurium* were not detected. Compared to 2018 to 2019, there was an increase in resistance percentages to most antibiotics tested.

The resistance rates of *E. coli* from chicken farms have been relatively stable since 2018. A steadily decreasing trend in resistance to nalidixic acid, and gradual increases in ciprofloxacin and ceftazidime resistance were observed. In 2021, there were increases in the proportion of MDR *E. coli* isolated from local chicken and ruminant farms. In contrast, *E. coli* from quail farms showed declining trends in resistance to several antibiotics.

FOOD PRODUCTS

In 2020 and 2021, 77.3% of all *Salmonella* isolated from imported chilled and frozen chicken meat products were found to be MDR. Isolates from frozen chicken meat products were more likely to be MDR than those from chilled chicken. An overall increase in %R for most antibiotics was observed, compared to rates last reported. Surveillance along the food supply chain found that ESBL *E. coli* (ESBL-Ec) were found more frequently in meat products sampled at retail than at points of import, processing (food establishments) or source, suggesting the possibility of extraneous contamination along the supply chain. Among different types of food products, ESBL-Ec was most frequently isolated from poultry products, with detection rates over 50% in 2020 and over 30% in 2021. In comparison, ESBL-Ec in pork and pork products ranged from 4.1% to 8.8%.

ENVIRONMENT

A study of six recreational beaches across Singapore found no ESBL-Ec in beach water sampled between Northeast Monsoon and inter-monsoon seasons in March 2021. Ceftriaxone-resistant bacteria were isolated but none were *E. coli*. However, a separate study of coastal waters and local waterways recovered ESBL-Ec from the water samples collected during the inter-monsoon (October 2021), Northeast monsoon (March 2022) and Southwest monsoon (August 2022) seasons. Levels on *E. coli* and *Enterococcus* spp in all sampling sites of both studies were well within international standards.

Introduction

Surveillance is one of five core strategies of Singapore's National Strategic Action Plan on Antimicrobial Resistance (AMR) (see Textbox 1). Surveillance of AMR and antimicrobial utilisation (AMU) provide important data for monitoring trends, assessing risks, guiding policy decisions and measuring their impact.

The *One Health Report on Antimicrobial Utilisation and Resistance, 2017*¹, was published in 2019 as a first step towards an integrated surveillance system in Singapore. In 2021, the second report (for data up to 2019) updated findings from existing surveillance programmes and included new data on antimicrobial sales for human use, WHO GLASS pathogens, and indicator AMR organisms in local dairy farms, the food production chain and in natural and man-made environments. Since then, national surveillance has been extended into the private acute care and public primary care settings. Several studies were also undertaken by agencies to examine ESBL *E. coli* (ESBL-Ec) in the food chain and environment, as well as AMR in two new populations: free-roaming dogs and wildlife. This third report updates on key findings in the human, animal, food and environment sectors up to 2021. With the continued expansion of AMR surveillance programmes, data from key populations and surveillance sites will contribute towards the formulation of science- and risk-based targets to further drive AMR control efforts.

Surveillance structure

National AMR and AMU surveillance and monitoring programmes in Singapore are implemented by the ministries and national agencies responsible for human health, animal health, food and environment. These sectors are in turn supported by participating hospitals, laboratories and other parties providing data to the relevant sector authorities. Data sharing and reporting across sectors is centrally coordinated under the AMR Workgroup (Figure 1).

Human health – Surveillance has been instituted in public hospitals since 2011, and in private hospitals since 2017. The incidence of priority drug-resistant organisms and utilisation of important antimicrobials are routinely monitored by all acute care hospitals in Singapore under a national programme overseen by the National Antimicrobial Resistance Control Committee (NARCC; Textbox 2). Antibiotic susceptibility testing (AST) is carried out by the hospitals' microbiology laboratories, while the National Centre for Infectious Disease (NCID)'s National Public Health Laboratory serves as the national reference laboratory for examining new and emerging resistance. Data analysed and compiled by NARCC are reported to the Ministry of Health (MOH), and provided to individual hospitals on a yearly basis for monitoring and control. Singapore also participates in the Global Antimicrobial Surveillance System (GLASS), which collects AMR data on the WHO priority pathogens¹ in humans. There are also on-going national surveillance programmes for HIV, gonorrhea and tuberculosis which are currently beyond the scope of this report.

¹ *E. coli*, *K. pneumoniae*, *A. baumannii*, *S. aureus*, *Streptococcus pneumoniae*, *Salmonella* spp, *Shigella* spp, *Neisseria gonorrhoeae*

Animal Health – The monitoring of priority drug-resistant organisms in local poultry and ruminant farms is currently undertaken by the National Parks Board (NParks) and the Singapore Food Agency (SFA). NParks' Centre for Animal & Veterinary Sciences (CAVS) conducts active AMR surveillance in free-roaming dogs and passive AMR surveillance in wildlife, sick companion and aquatic animals. Data on antimicrobial sales for veterinary use are collected yearly by NParks and reported to the World Organisation for Animal Health (WOAH) on the global database on ANImal antiMicrobial USE (ANIMUSE).

Food – Monitoring of antimicrobial-resistant foodborne bacteria has been in place since 2010 as part of the national food safety surveillance programme, with a focus on *Salmonella* spp. Surveillance and antimicrobial susceptibility testing of AMR foodborne bacteria are conducted by SFA's National Centre for Food Science (NCFS). SFA's efforts for AMR monitoring in the food chain includes establishing baseline data on the prevalence of resistance to antimicrobial agents in commensal bacteria and food-borne pathogens based on stratified random sampling of target food across the food chain particularly at import, slaughterhouses, local farms and retail levels, as well as food-producing animals intended for consumption.

Environment – National surveillance programmes for drug-resistant organisms in the environment are jointly developed by NEA and PUB. NEA conducts studies to examine the presence and patterns of resistance in environmental bacteria found in various natural and man-made environments. PUB conducts studies to examine the presence and risks of antibiotic resistant bacteria and genes in urban waters in collaboration with local research institutions.

National Coordination – The One Health AMR Workgroup (AMRWG, see Textbox 1) coordinates the sharing of AMR and AMU data across sectors. This work is supported by the AMR Coordinating Office (AMRCO) of the NCID. The longer-term goal is to build a more integrated approach for national AMR and AMU surveillance in Singapore that would better elucidate the prevalence and transmission routes of AMR pathogens across sectors.

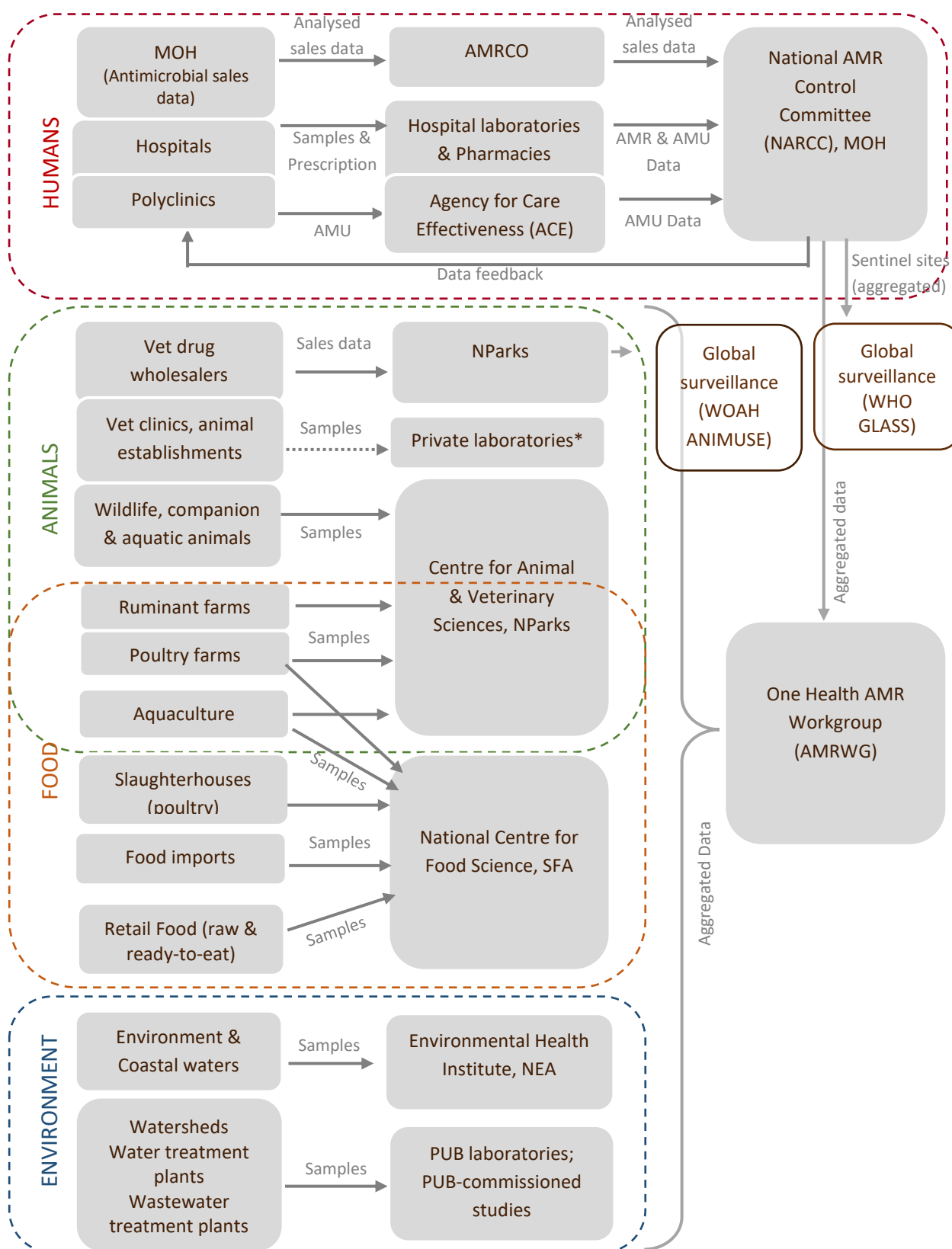
Textbox 1

Singapore's National Strategic Action Plan on AMR

The National Strategic Action Plan (NSAP) was launched in November 2017. It aims to reduce the emergence and prevent the spread of drug-resistant organisms in Singapore through five core strategies: Education, Surveillance and Risk Assessment, Research, Prevention and Control of Infection, and Optimisation of Antimicrobial Use. Implementation is overseen by the One Health AMR Workgroup (AMRWG), a multi-sectoral committee comprised of representatives from the Ministry of Health (MOH), Health Promotion Board (HPB), National Environment Agency (NEA), National Parks Board (NParks), PUB the National Water Agency and the Singapore Food Agency (SFA). The AMRWG is supported by the Antimicrobial Resistance Coordinating Office (AMRCO) of the National Centre for Infectious Diseases (NCID). Singapore's NSAP may be found at:

<https://www.ncid.sg/About-NCID/OurDepartments/Antimicrobial-Resistance-Coordinating-Office/Documents/National%20Strategic%20Action%20Plan%20on%20Antimicrobial%20Resistance.pdf>

Figure 1. AMR & AMU surveillance and monitoring in Singapore



*Veterinary clinics may choose to send their samples to the national laboratory (CAVS) or to private laboratories. Data from these private laboratories currently do not contribute to national surveillance.

Human and Animal Populations

Singapore's population in 2021 was 5.45 million². The population is served by 19 acute care hospitals³ of which 10 are public facilities that account for 80% of hospital admissions. Another nine private acute care hospitals⁸ account for the remaining admissions that includes a sizeable proportion of international patients. In Singapore, primary care is provided through 23 public polyclinics and 2,410 general practitioner clinics³ run by private sector general practitioners (GP). The polyclinics meet about 20% of the total primary care demand⁴.

Pet ownership in Singapore is also on the rise. The number of dogs licensed by the NParks increased from 59,000 in 2011 to 87,000 in 2021. There are currently 104 veterinary centres registered with NParks. The non-food producing animal population consists of approximately 110,900 registered dogs and cats, 1,400 horses, and 7,200 pet birds.

As a highly urbanised country with low local agricultural production, Singapore is highly dependent on imports for its food requirements. More than 90% of food is imported from over 170 countries⁵. The remaining 10% are locally produced. Singapore has a small, but thriving and increasingly important food fish aquaculture industry, which currently accounts for about 8% of local food fish consumption and has been rising through the years. As Singapore aims to produce 30% of the population's nutritional needs locally by 2030, it is envisaged that the local aquaculture sector would continue to develop and expand. Three chicken layer farms producing approximately 30% of local consumption of table eggs are currently the most significant livestock establishments. The population of food animals in Singapore and production outputs are presented in Table 1.

Table 1. Production of food animals, eggs and fish, Singapore 2021

Type	No. of farms	Total population	Production (% national consumption)
Chicken layers	3	≈3,000,000	643.7 million eggs (30.5%) ⁶
Quail layers	2	155,000	≈30 million eggs (100%)
Dairy cattle	2	121	-
Dairy goats	1	848	-
Farmed fish	111 sea-based ⁶ 24 land-based ⁶	Not applicable	4,900 tonnes (7.8%) ⁶

² www.singstat.gov.sg

³ www.moh.gov.sg/resources-statistics/singapore-health-facts/health-facilities, 2021

⁴ Primary Healthcare Services, www.moh.sg. Accessed on 29 Dec 2022

⁵ SFA annual report, 2021-2022

PART I. ANTIMICROBIAL UTILISATION

Antimicrobial Utilisation in Humans

Antimicrobial Utilisation Acute Care Hospitals

NARCC (Textbox 2) monitors the utilisation of important antimicrobial groups, such as broad-spectrum penicillins, third- and fourth-generation cephalosporins, fluoroquinolones and carbapenems. Public hospitals have been reporting AMU data since 2011, and private hospitals since 2017. This edition reports data beginning from 2017 when private hospital data became available; data on public hospital utilisation from 2011 to 2016 remain available in the earlier reports. This report includes the data from nine public and eight private acute care hospitals currently contributing AMU data to NARCC.

Antimicrobial Stewardship Programmes (ASP) were established in all public hospitals in 2011 with the aim of improving patient outcomes and optimising antimicrobial use through a system of audits and feedback. The rates of appropriate antibiotic prescribing and the acceptance rates of ASP interventions are monitored by MOH through NARCC. Under this programme, ASP pharmacists and infectious disease specialists work closely to promote the appropriate use of antimicrobials with the aim of preserving the effectiveness of antimicrobials for treatment and reducing the emergence of AMR in public hospitals.

Utilisation by acute care hospitals is typically reported as DDD per 1,000 inpatient days. DDD is calculated based on prevailing values published by WHO⁶; adjustments to DDD values by WHO should therefore be taken into consideration when interpreting AMU trends presented in DDD. These limitations notwithstanding, the use of DDD is a commonly accepted and practical way to measure antimicrobial consumption. The use of inpatient days as a denominator allows for a measurement of the incidence density (cases per 10,000 inpatient days) to be obtained and allows normalisation across hospitals of different sizes.

Textbox 2

National AMR Control Committee (NARCC)

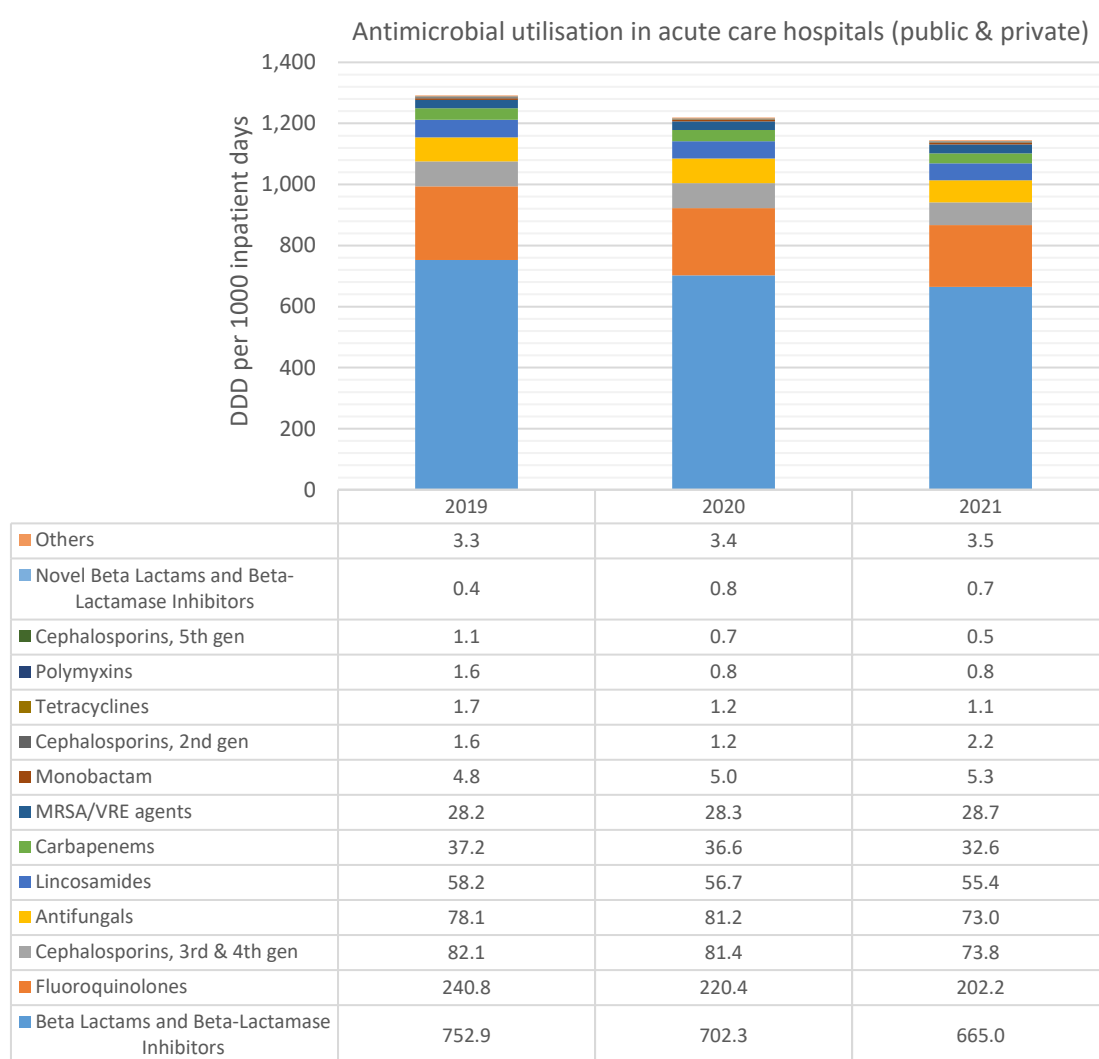
The National AMR Control Committee (NARCC) is appointed by the Ministry of Health (MOH) to oversee surveillance in the human health sector. NARCC is represented by all acute care hospitals and assists MOH in developing strategies to control the emergence and spread of AMR. NARCC is supported by two expert panels: The National Antimicrobial Resistance Expert Panel (NAREP), and the National Antimicrobial Stewardship Expert Panel (NASEP). The expert panels, comprising microbiologists, infectious disease physicians and pharmacists from public healthcare institutions, advise on issues related to surveillance of antimicrobial resistant organisms and antimicrobial utilisation. NARCC collects AMR and AMU data every 6 months from hospital laboratories and antibiotic stewardship teams. Data compiled and analysed by NARCC are provided to hospitals' management and MOH on a yearly basis. The data are used to monitor trends in hospitals and implement control measures where appropriate.

⁶ https://www.whocc.no/atc_ddd_index/

In 2021, NARCC monitored the utilisation of 45 antimicrobials under 14 NARCC-defined groups (Appendix I.I). This represents an increase of 13 antimicrobials since the last report. Twelve antimicrobials were introduced in 2019: amoxicillin-clavulanate, oral; ampicillin-sulbactam, IV and oral; ceftolozane-tazobactam, IV; aztreonam, IV; ceftaxime, IV; cefixime, oral; cefoperazone, IV; cefotaxime, IV; ceftibuten, oral; ceftaroline, IV; teicoplanin, IV; and tedizolid, IV and oral. In 2020, the monitoring of ceftazidime-avibactam, IV, was introduced.

In 2021, the five groups of antimicrobials most utilised by public and private acute care hospitals in Singapore were beta-lactams and beta-lactamase inhibitors, fluoroquinolones, third- and fourth-generation cephalosporins, antifungals and lincosamides (clindamycin) (Figure 2).

Figure 2. Antimicrobial utilisation in acute care hospitals (public and private combined) in Singapore, for all antimicrobials under monitoring from 2019 - 2021



Note: Not all antimicrobials used in hospitals are monitored. The following antimicrobials were introduced for monitoring in 2019 - **Beta lactams and Beta-Lactamase Inhibitors:** Amoxicillin-clavulanate, oral and ampicillin-sulbactam, IV; **Novel Beta lactams and Beta-Lactamase Inhibitors:** oral ceftolozane-tazobactam, IV; **Monobactam:** aztreonam, IV; **Cephalosporin, second generation:** ceftaxime, IV; **Cephalosporin third and fourth generation:** cefixime, oral; cefoperazone, IV; cefotaxime, IV; ceftibuten, oral; **Cephalosporin, fifth generation:** ceftaroline, IV; **MRSA/VRE agents:** teicoplanin, IV; tedizolid, IV and oral. In 2020: **Novel Beta lactams and Beta-Lactamase Inhibitors:** Ceftazidime-avibactam, IV. The full list of antimicrobials monitored may be found at Appendix I.

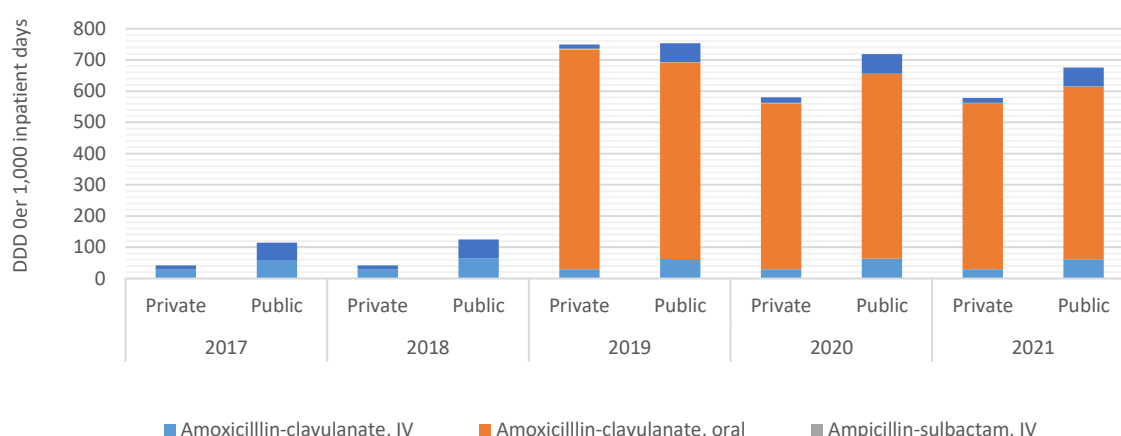
Overall utilisation of antimicrobials by acute care hospitals declined from 2019 to 2021. The COVID-19 pandemic as a contributing factor to this trend has not been assessed; further post-pandemic monitoring may shed light on its possible impacts.

Within each group of antimicrobial drugs, some differences between public and private hospitals with respect to specific utilisation were observed and are described below. It is not the intent of this report to examine the reasons for these differences, recognising that these are multi-factorial, including but not limited to differences in patient case-mix, hospital drug formulary and operating models. In addition, data sources are not uniform across all hospitals. Attempts to draw conclusions from this limited dataset alone should, therefore, be avoided.

Beta-lactams and beta-lactamase inhibitors

Beta-lactams and beta-lactamase inhibitors were the most utilised group in both public and private acute care hospitals. Within this group of antimicrobials, oral amoxicillin-clavulanate was the most heavily used of all antimicrobials monitored in 2021. Average utilisation of oral amoxicillin-clavulanate was slightly higher in public hospitals than in private hospitals, at 552.9 DDD per 1000 inpatient days in public hospitals and 531.6 DDD per 1,000 inpatient days in private hospitals (Figure 3).

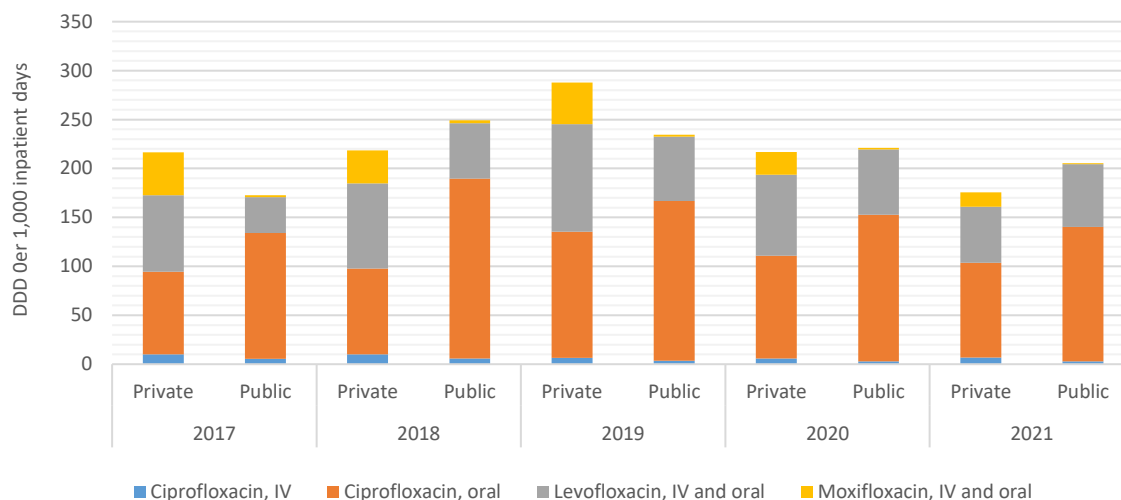
Figure 3. Utilisation of beta-lactams and beta-lactamase inhibitors in public and private acute care hospitals, 2017-2021



Notes: Amoxicillin-clavulanate, oral; ampicillin-sulbactam, IV and oral were included for monitoring from 2019.

Fluoroquinolones

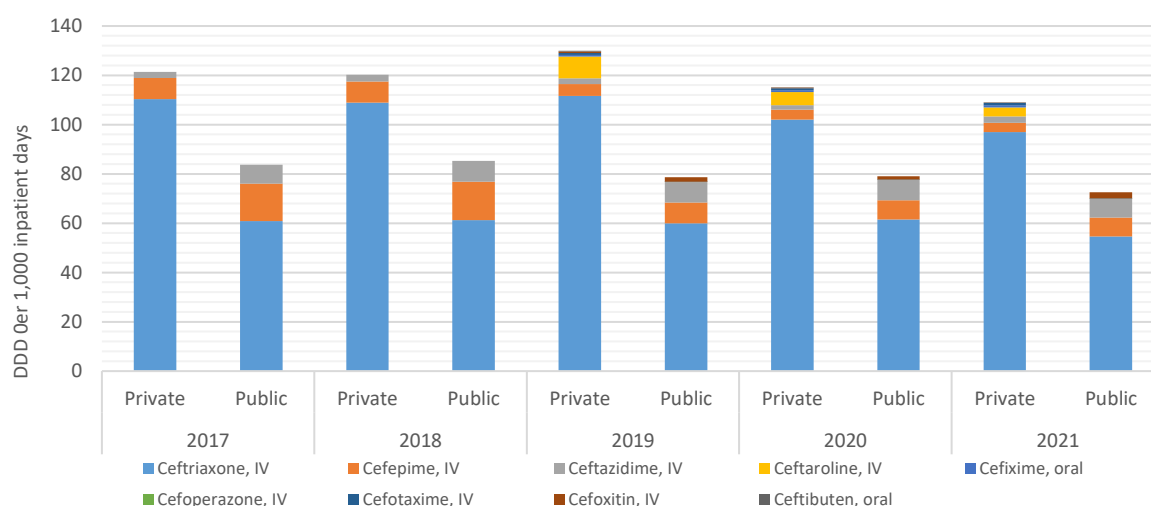
Fluoroquinolones were the next most utilised group of antimicrobials, with oral ciprofloxacin being the most used, followed by levofloxacin. Oral ciprofloxacin utilisation was higher in public hospitals than private hospitals, at 137.3 DDD and 97.1 DDD per 1,000 inpatient days respectively. Levofloxacin utilisation was higher in private hospitals than in public hospitals up to 2020; however, in 2021, levofloxacin utilisation in private hospitals was lower than that of public hospitals (56.9 vs 64.2 DDD per 1,000 inpatient days). Overall, the use of fluoroquinolones in both public and private hospitals declined in 2020 and 2021, mostly driven by reduction in oral ciprofloxacin use (Figure 4).

Figure 4. Utilisation of fluoroquinolones in public and private acute care hospitals, 2017-2021

Note: Outpatient utilisation was included in public hospitals data from 2018.

Cephalosporins

Third- and fourth-generation cephalosporins were the third most utilised group, of which ceftriaxone was the most utilised cephalosporin antibiotic. Private hospital utilisation exceeded that of public hospitals, at 97.0 DDD per 1,000 inpatient days compared with 54.7 DDD per 100 inpatient days in public hospitals in 2021. Ceftriaxone, a fifth-generation cephalosporin, was used mainly in the private sector, though utilisation was generally low and on a declining trend (Figure 5).

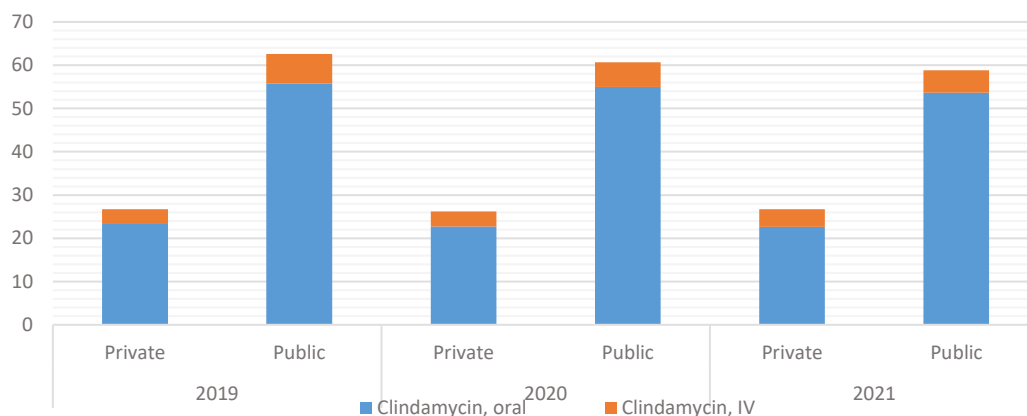
Figure 5. Utilisation of cephalosporins in public and private hospitals, 2017-2021

Note: Revised DDD assignment for IV cefepime from 2g to 4g in 2019 may have contributed to observed reduction in utilisation for that year.

Lincosamides

Monitoring of lincosamides, specifically clindamycin, was introduced in 2019. Utilisation of oral clindamycin was higher in public hospitals than in private hospitals, at 53.7 compared to 22.7 DDD per 1,000 inpatient days respectively (Figure 6).

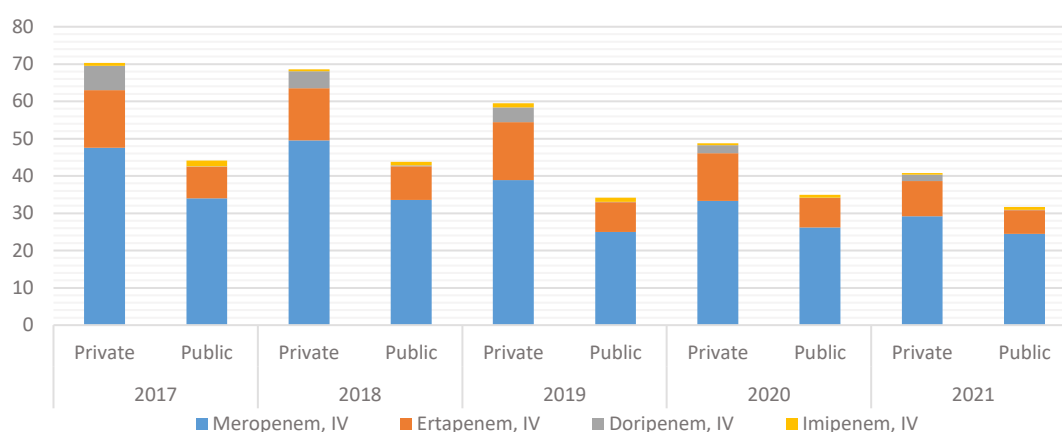
Figure 6. Utilisation of lincosamides in public and private hospitals, 2019 – 2021



Carbapenems

Both private and public hospitals demonstrated declining trends in carbapenem utilisation from 2017 to 2021. The use of carbapenems was higher in private hospitals than public hospitals (Figure 7). Meropenem remains the most widely used carbapenem. In 2021, utilisation of meropenem in private hospitals was 29.2 DDD per 1,000 inpatient days while public hospital utilisation was 24.4 DDD per 1,000 inpatient days. Doripenem is used mainly in private hospitals, while imipenem has limited utilisation in both public and private hospitals.

Figure 7. Utilisation of carbapenems in public and private acute care hospitals, 2017-2021

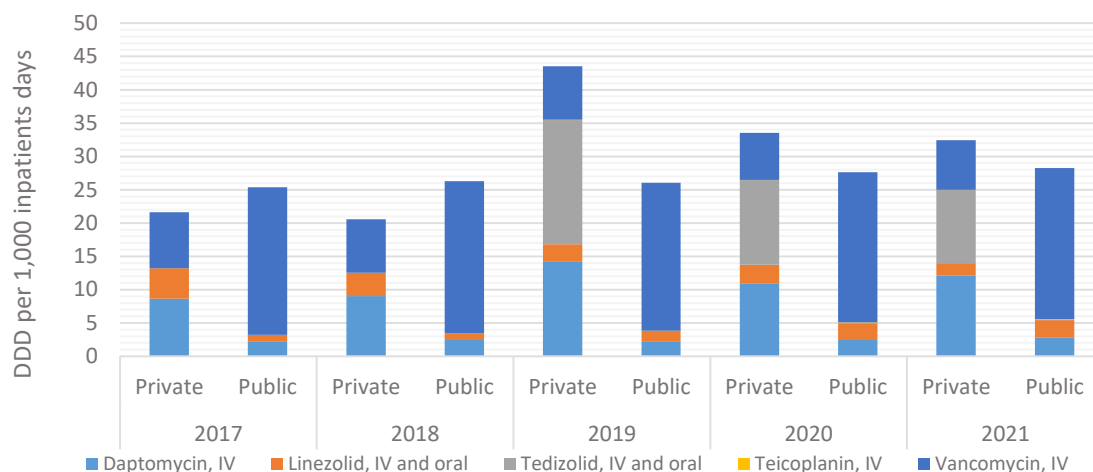


MRSA and VRE agents

This group includes vancomycin, daptomycin, linezolid, tedizolid and teicoplanin. Overall utilisation of this group of antimicrobials was higher in private than public hospitals (Figure 8). Daptomycin and

tedizolid were used more by private hospitals while vancomycin was used more widely by public hospitals. Tedizolid and teicoplanin were introduced for monitoring in 2019; tedizolid was not used in public hospitals during this period of reporting, while teicoplanin was not used by any hospital.

Figure 8. Utilisation of MRSA & VRE agents in public and private acute care hospitals, 2017-2021

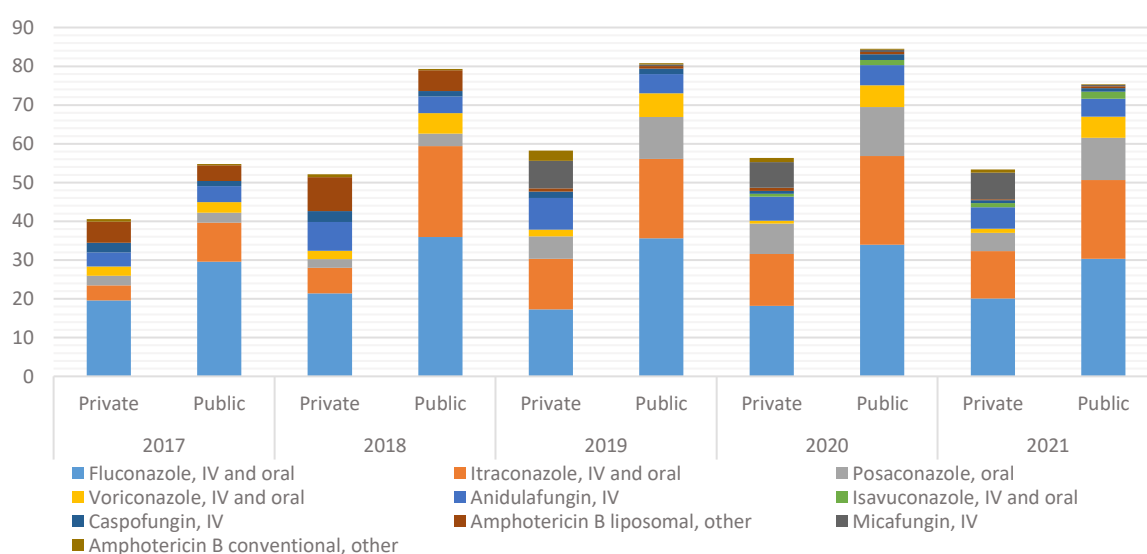


Note: The use of teicoplanin and tedizolid were monitored from 2019

Antifungals

Fluconazole was the most utilised antifungal from 2017 to 2021. Overall utilisation of antifungals was higher in public than private hospitals (Figure 9). Miconazole, included for monitoring in 2019, was used mainly in private hospitals, while the use of itraconazole and fluconazole was higher in public hospitals. As WHO does not set a specific DDD value for liposomal amphotericin B, a local DDD of 300mg was applied in 2019, accounting for the apparent reduction in the utilisation of liposomal amphotericin B after 2018.

Figure 9. Utilisation of antifungals in public and private acute care hospitals, 2017-2021



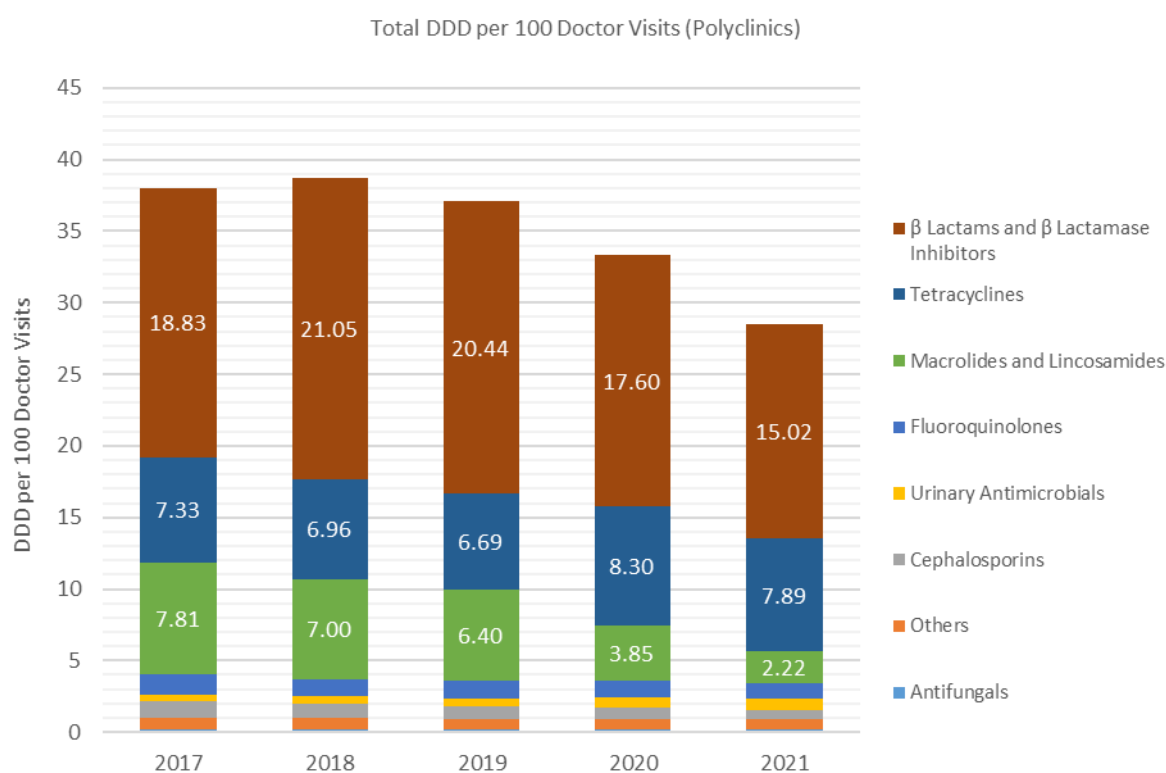
Note: 1. In 2019, DDD of oral posaconazole was updated from 0.8g to 0.3g; DDD of liposomal amphotericin B was updated from 35mg to a local DDD of 300mg. 2. Data on Miconazole IV was collected from 2019; data on isavuconazole, IV and Oral was collected from 2020.

Antimicrobial utilisation in primary care settings (polyclinics)

Polyclinics in Singapore serve as public sector primary care clinics. In 2021, there were a total of 23 polyclinics distributed throughout the country providing acute and chronic care to approximately 20% of the outpatient community. The collection of AMU data from polyclinics was initiated in 2019 for antimicrobials utilised in 2018. Data collected were based on self-reporting by the various polyclinics. Utilisation rates were measured in DDD per 100 doctor visits, under eight groups of antimicrobials (Figure 10).

A decline in the utilisation of oral antimicrobials from 2018 to 2021 was observed (Figure 10). In 2021, beta-lactams and beta-lactamase inhibitors (15.02 DDD per 100 doctor visits) were the most utilised antimicrobial group, followed by tetracyclines (7.9 DDD per 100 doctor visits) and macrolides and lincosamides (2.22 DDD per 100 doctor visits). Overall, these three antimicrobial groups with the highest utilisations in the polyclinics have remained consistent across the years.

Figure 10. Antimicrobial utilisation in Singapore's polyclinics, from 2017 – 2021



Beta Lactams and Beta-Lactamase Inhibitors: Phenoxymethylpenicillin (penicillin v), Amoxicillin-Clavulanate, Amoxicillin, Cloxacillin; **Tetracyclines:** Doxycycline, Tetracycline; **Macrolides & Lincosamides:** Azithromycin, Clarithromycin, Clindamycin, Erythromycin ethylsuccinate/stearate; **Fluoroquinolones:** Ciprofloxacin; **Cephalosporins:** Cefalexin, Cefuroxime; **Urinary Antibiotics:** Nitrofurantoin; **Other Antibiotics:** Metronidazole, Co-trimoxazole; **Antifungal:** Nystatin

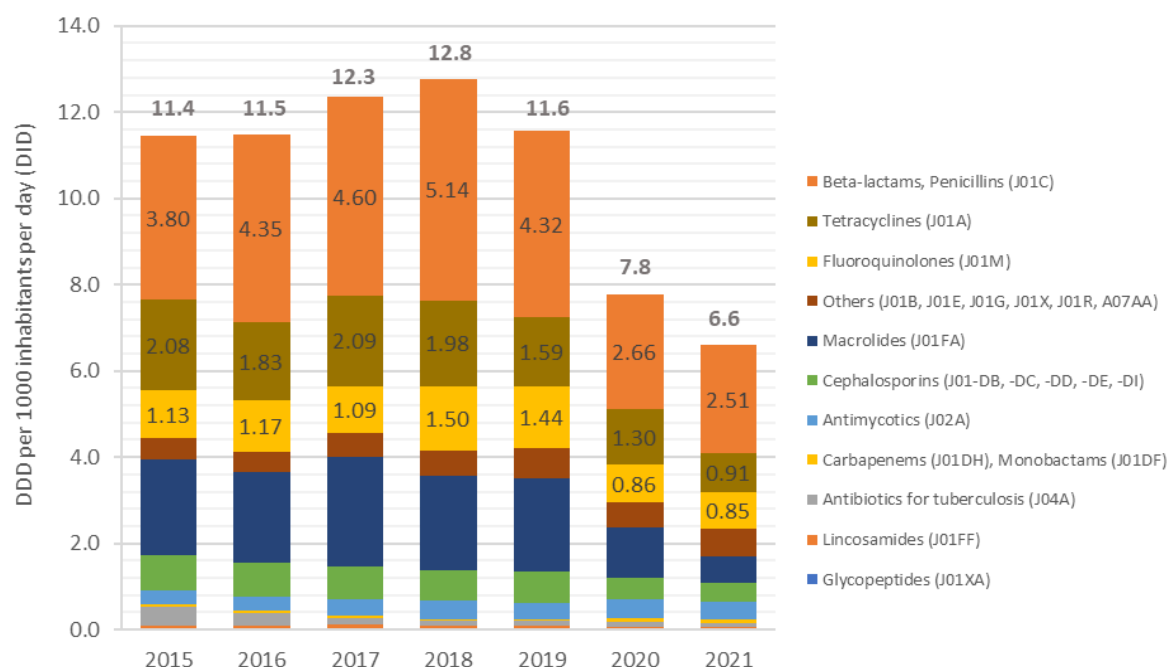
Data Source: MOH Singapore

Antimicrobial Sales for Human use

Sales data⁷ serve as an additional reference to antimicrobial utilisation trends. Sales data is a more readily available form of data and may be used as a general reference to antimicrobial consumption trends. National antimicrobial consumption is typically reported as DDD per 1,000 inhabitants or DID (**Error! Reference source not found.** I: Methodology). The main category of antimicrobials tracked are those classified under Anatomical Therapeutic Chemical (ATC) code J01 – Antibacterials for systemic use.

In 2021, the sales of beta-lactams, penicillins (mainly amoxicillin-beta lactamase inhibitors) remained the highest. This was followed by tetracyclines (mainly doxycycline) and fluoroquinolones (mainly ciprofloxacin). We observed a substantial reduction in total reported sales volume of J01 antibiotics in 2020 and 2021, falling to 6.6 DID in 2021 (Figure 11), largely due to a reduction in sales to private sector outpatient clinics (Figure 12). Private sector clinics include all private dispensing clinics (GPs and specialists) and specialists dispensing clinics within private hospitals. Sales to private sector outpatient clinics in 2020 and 2021 declined by approximately 50% from sale volumes reported in 2019. Further monitoring post-pandemic would enable us to assess if the COVID-19 pandemic was a contributing factor to this decline. Nevertheless, private sector outpatient clinics sales remained the highest among the four main channels namely, clinics, pharmacy, hospitals and others (Figure 12).

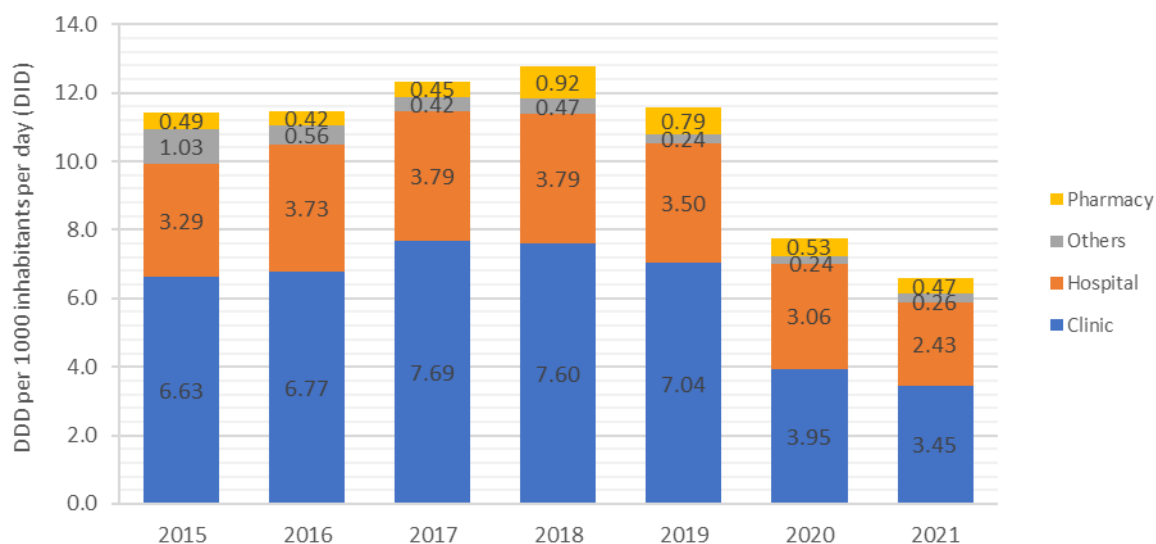
Figure 11. Total Sales of antimicrobials, 2015 – 2021, by antimicrobial class (in DDD per 1000 inhabitants; DID)



⁷ Data source: MOH Singapore

Note: DID of the three most sold classes of antimicrobials are indicated within the columns. The total annual reported sales for the year in DID are indicated above each column. Data: IQVIA National Sales Audit. Source: MOH Singapore.

Figure 12. Total Sales of antimicrobials by sales channel 2015 – 2021 (in DDD per 1000 inhabitants; DID)



Channels: **Pharmacy** - All pharmacies e.g. chained pharmacies and independent; **Others** - Polyclinics and other institutes like nursing homes, social service centres, community hospitals; **Hospital** - Restructured/public hospitals, private hospitals (excluding dispensing clinics in private hospitals); **Clinic** - All private dispensing clinics (GPs and Specialists) including specialists dispensing clinics within private hospitals. Data: IQVIA National Sales Audit. Source: MOH Singapore.

The data reported here were based solely on private market research sales quantities and should be interpreted in context: The total DDD per 1000 inhabitants/day (DID) calculated from this dataset may underestimate true national consumption due to its incomplete coverage of generic antimicrobials. Furthermore, sales data may not correlate well with utilisation data, since not all antimicrobials sold may be utilised within the period of sales data reporting. Nevertheless, in the absence of national-level utilisation data, sales data enables the monitoring of trends within different healthcare settings. As we increase the comprehensiveness of local AMU data collection, we will be better able to draw associations between sales and consumption/utilisation patterns.

Antimicrobial Utilisation in Animals

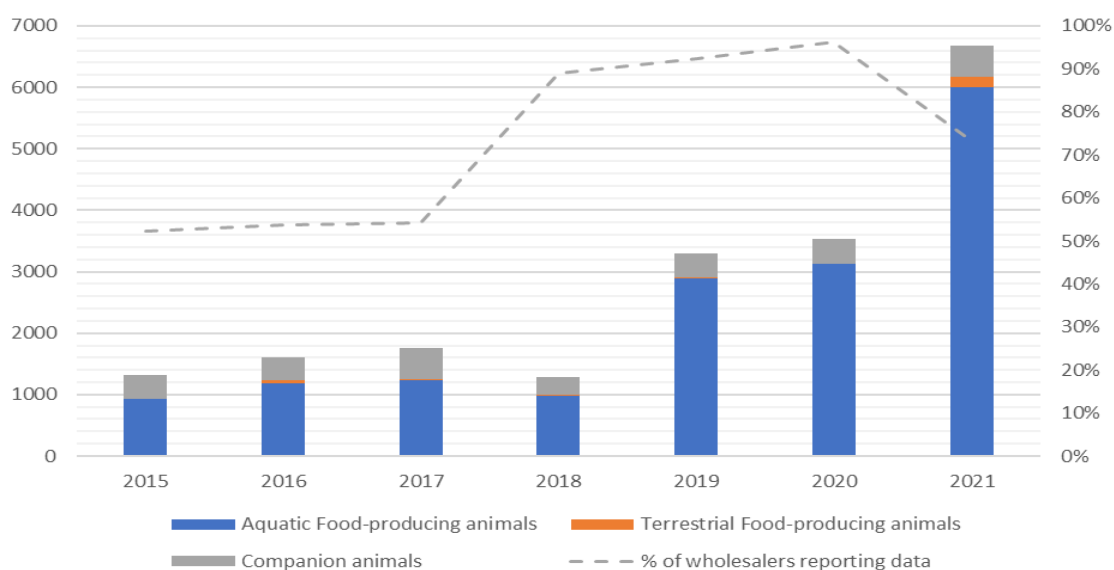
Antimicrobials serve an important role in veterinary medicine in the prevention and treatment of animal diseases. Preservation of antimicrobials is crucial to maintain drug efficacy and minimise both food security and animal health and welfare concerns in the event of a disease outbreak. In Singapore, the use of antimicrobials for the purpose of growth promotion is prohibited. Additionally, certain antimicrobials and substances, such as chloramphenicol, polymyxins, avoparcin, beta-agonists and nitrofurans, are prohibited for use in local food-producing animals and in animal feed. The use of antimicrobials in local commercial chicken farms throughout the laying period is also disallowed to prevent the presence of antimicrobial residues in table eggs intended for human consumption. These prohibitions are supported by a robust residue monitoring programme and post-market surveillance.

Antimicrobial Sales for Veterinary Use

Singapore has been reporting data on sales of antimicrobials for animal use to the World Organisation for Animal Health (WOAH) annually since 2015. Sales data collected serve as a proxy for antimicrobial utilisation in animals. Overall, the total quantity of antimicrobial sales recorded increased from 1318.2 kg in 2015 to 6681 kg in 2021 (Figure 13), largely due to more wholesalers reporting data through an expanded survey base.

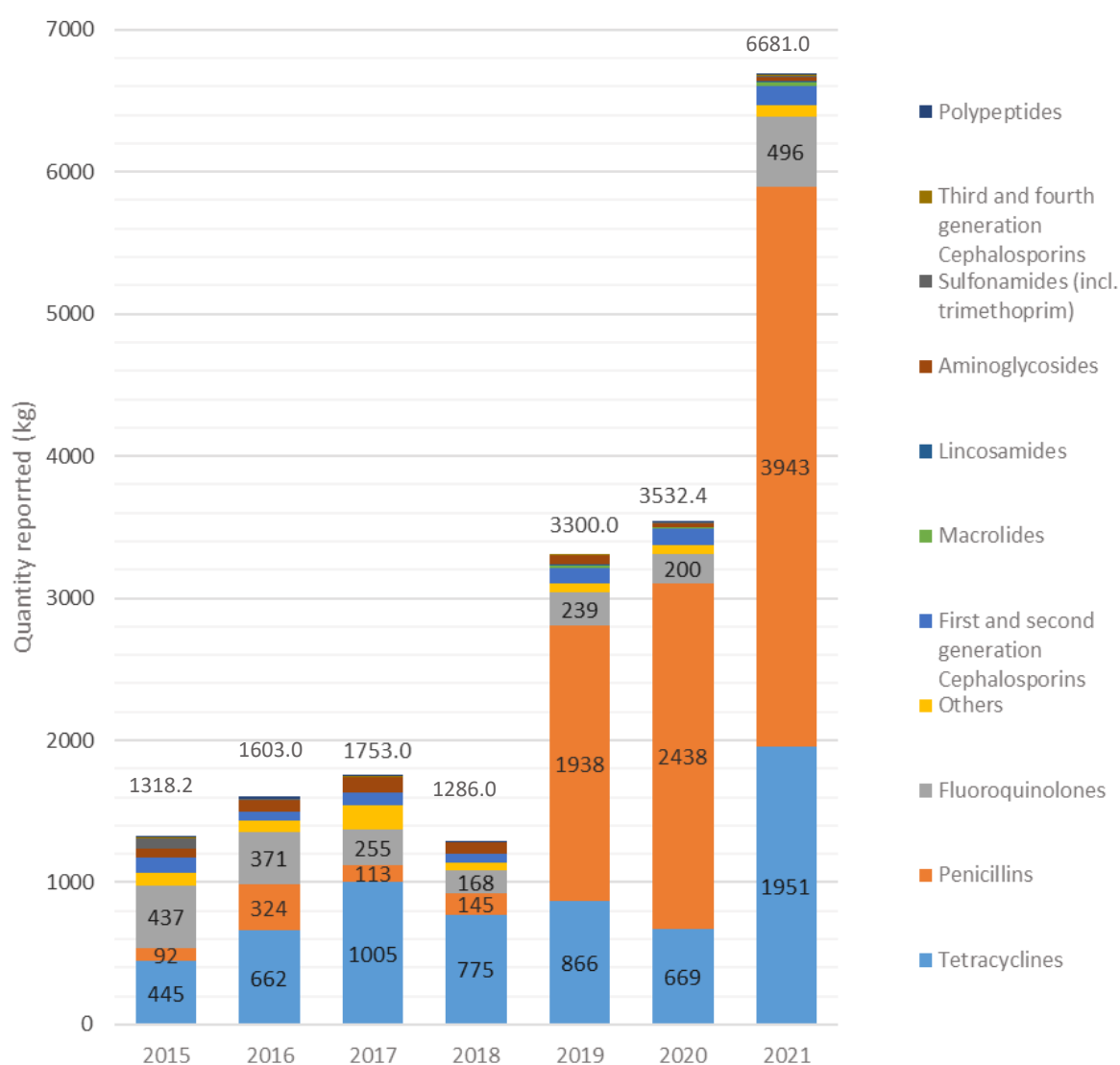
From 2015 to 2020, NParks collected sales data through a voluntary survey of wholesalers supplying human antimicrobials to the animal sector for off-label use, of which 26 out of 27 (~96%) surveyed in 2020 had provided their responses. In 2021, NParks expanded its survey to include additional companies supplying veterinary antimicrobials to the animal sector so as to improve representativeness of AMU data collected. Of these companies, 41 out of 56 companies (~73%) surveyed in 2021 provided their responses. Hence, while the proportion of companies which responded decreased from 2020 to 2021, the absolute number of companies which responded increased, contributing to the increased sales reported in 2021.

Figure 13. Annual reported sales (kg) of antimicrobial drugs in animal sector, 2015 – 2021



Tetracyclines, fluoroquinolones and penicillins were the most reported antimicrobial drugs for veterinary use from 2015 to 2021 (accounting for about 74% to 96% of total antimicrobial quantity) (Figure 14). Reported quantities of tetracyclines increased year-on-year from 2015 to 2021, attributed largely to increased sales to the aquaculture sector. Fluoroquinolone sales decreased from 437 kg in 2015 to 200 kg in 2020 and increased to 496 kg in 2021. Penicillin sales have generally increased from 2015 to 2021, with a significant increase from 2019 onwards due to the need to manage multiple disease outbreaks in farmed food fish. No antimicrobials were sold for non-therapeutic uses (e.g., growth promotion) from 2015 to 2021.

Figure 14. Annual reported sales (kg) of antimicrobial drugs in the animal sector, by antimicrobial class, 2015 to 2021



Note: Numbers above bars denote total quantities reported for the year in kg.

Aquaculture

Majority of fish farms employ traditional farming methods, although there is a small but growing segment of progressive farms which are more technology driven. Of the food production sectors in Singapore, the aquaculture industry consumes the most antimicrobial drugs (by weight) each year amongst the animal sectors, accounting for about 70% to 90% of total antimicrobial sales in the animal sectors from 2015 to 2021.

Aquaculture is a growing food production sector for Singapore, given Singapore's "30-30 vision" to build our local agrifood industry's capability and capacity to sustainably produce 30% of Singapore's nutritional needs by 2030. Amongst the animal sectors, this sector accounts for the highest use of tetracycline, first and second generation cephalosporins, and fluoroquinolones (enrofloxacin) (Figure 15). Antimicrobial usage is further compounded by the limited availability of tropical food fish vaccines worldwide, and reduced cost-effectiveness of vaccination for smaller holdings.

Terrestrial livestock

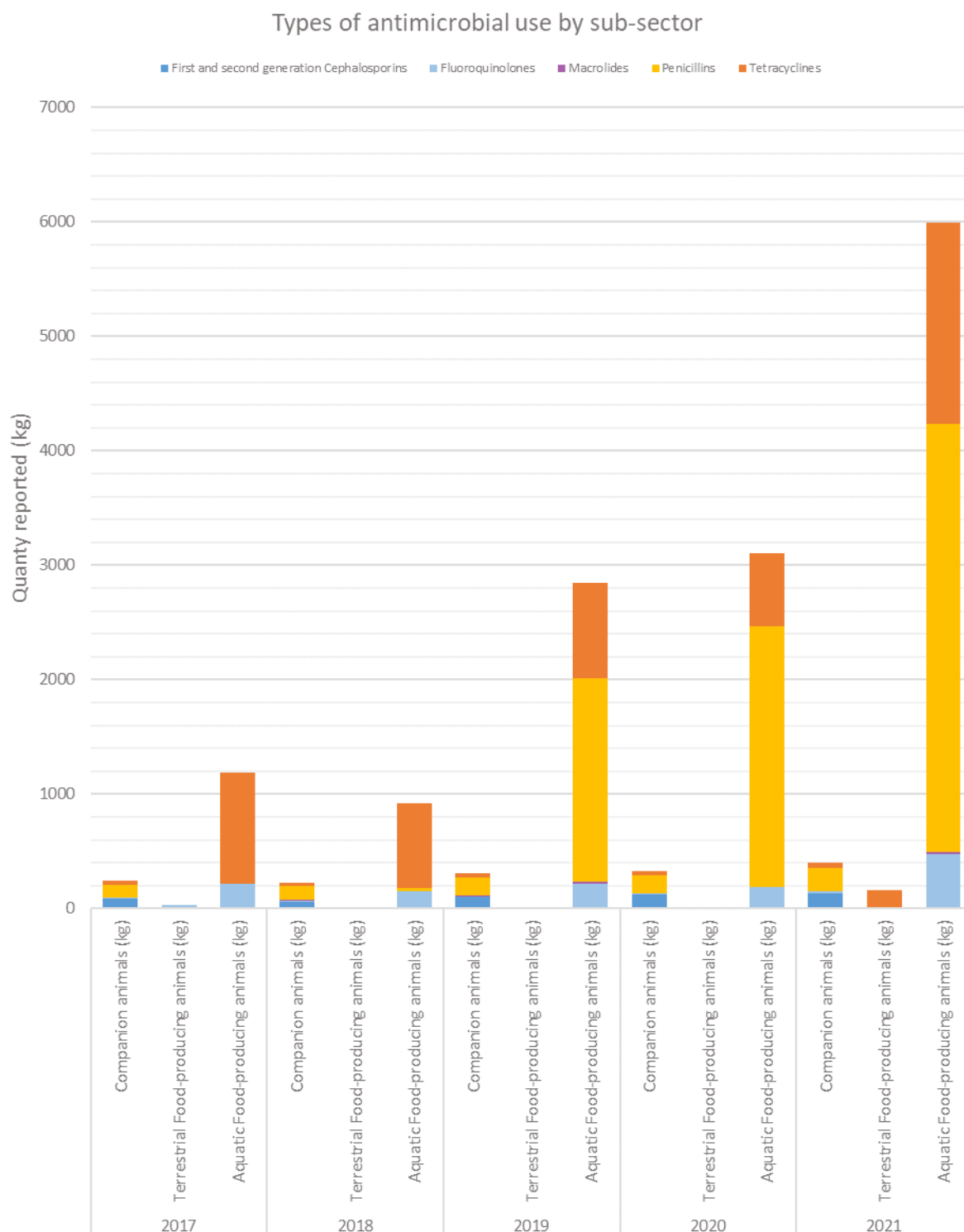
AMU in terrestrial livestock remains low despite the highly intensive nature of local chicken layer farms. This is reflective of the mature state of the chicken layer industry here, characterised by adherence to good animal husbandry practices, effective biosecurity measures to prevent disease introduction, routine vaccination programmes for disease prevention and good compliance to antibiotic restrictions.

Companion animals

AMU in the companion animal sector has generally remained stable over the years, despite the growing number of companion animals. The number of licensed dogs in Singapore has increased by almost 13% from about 62,000 in 2015 to 87,000 in 2021 and the cat population is also increasing. The number of veterinarians in companion animal practice and the number of companion animal veterinary clinics have also correspondingly increased to 104 in 2021.

The stable AMU in the companion animal sector, despite the growing pet population and industry, suggests responsible prescribing and use of antimicrobials in this sector. Other contributing factors include good animal husbandry and management practices of animal owners and caregivers, and improved uptake and awareness of preventative care, such as vaccination, anti-parasitic treatments, and regular health checks.

Figure 15. Quantities of antimicrobials per animal sector for the 5 classes with highest reported AMU, from 2017 to 2021.



Conclusions and Steps Forward

Since our last report, data collection on antimicrobial consumption has expanded into the public primary care sector, through the monitoring of polyclinic antimicrobial utilisation. As reliable estimates of nation-wide antimicrobial utilisation are still lacking, sales volumes continue to serve as the main source of national consumption estimates for the human and animal health sectors. Despite its limitations, sales data provide useful insight as to where antimicrobials are most used and will continue to be tracked. Given the limited agriculture sector here, sales for human use in Singapore exceed that for animal use. In the human health sector, sales to private outpatient clinics are approximately twice that for public and private inpatient use. For the animal sector, the aquaculture industry in Singapore continues to be the main consumer of antimicrobials.

Sales data are insufficient to illuminate consumption patterns and volumes at the clinic (both human and veterinary) or farm level. Annual sales trends should therefore be interpreted with caution as they do not necessarily correspond with utilisation. The collection of comprehensive utilisation data remains the most valuable for monitoring consumption and guiding the development of targeted, effective strategies and interventions. Work is underway in the human health sector to collect data on antimicrobial utilisation in the private primary care sector, i.e., from private general practitioners. Likewise, efforts are being made to collect data on antimicrobial utilisation at farm level.

The surveillance data show that there is need for continual education and engagement across sectors to reduce reliance on antimicrobials and ensure appropriate use. Ongoing national education initiatives and public engagement sessions have been conducted to raise awareness on the responsible use of antimicrobials in the human health and animal sectors. Antimicrobial stewardship programmes are actively promoted in healthcare institutions, while SFA and NParks continue to work with the farming sector to improve vaccination and health management programmes. NParks, in collaboration with the Singapore Veterinary Association, has published national guidelines on vaccination and prudent antimicrobial use for companion animals. Moreover, AMR is also included as a topic in NParks' veterinary licensing briefings and will eventually be incorporated into a new course on Responsible Pet Ownership (RPO) that NParks is currently developing for pet owners as well.

PART II. ANTIMICROBIAL RESISTANCE

Antimicrobial Resistance in Human Health

AMR surveillance in hospitals

NARCC collects data on seven important pathogens isolated from clinical samples from acute care hospitals: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterococcus faecium* and *Enterococcus faecalis*. In addition to specific pathogen-drug combinations, NARCC monitors the incidence density rates of *Clostridioides difficile*, which is associated with antimicrobial overuse (Refer to Appendix 1: Methodology for Table of drug-pathogen combinations and sample types). This report includes data from nine public and eight private acute care hospitals currently contributing AMR data to NARCC.

E. coli and *K. pneumoniae* are monitored for resistance to three important antibiotic classes:

- i. Ceftriaxone resistance (or an equivalent third-generation cephalosporin) is an indicator for extended-spectrum beta-lactamase (ESBL), and cephalosporinases. These resistance mechanisms usually result in patients needing treatment with carbapenems, which are very broad-spectrum, second- or later-line antibiotics.
- ii. Ciprofloxacin resistance is a marker for fluoroquinolone resistance and can potentially be correlated with widespread fluoroquinolone use in the community as well as in hospitals.
- iii. Carbapenem resistance (defined as meropenem or imipenem non-susceptibility) is an emerging concern because infections caused by carbapenem-resistant organisms typically require treatment with other last-line antibiotics. Resistance mechanisms include carbapenemase production, and a combination of ESBL or AmpC production with porin loss. Carbapenemases are beta-lactamases with the ability to hydrolyse penicillins, cephalosporins, monobactams and carbapenems.

P. aeruginosa is an opportunistic pathogen which can cause serious community-acquired and nosocomial infections and is of particular concern in neutropaenic patients. *P. aeruginosa* is also a relatively frequent coloniser of medical devices, such as in-dwelling catheters, and can harbour multiple antibiotic resistance mechanisms. *P. aeruginosa* is monitored for resistance to carbapenems (imipenem or meropenem).

A. baumannii is an important cause of nosocomial infections including pneumonia, urinary tract, bloodstream, catheter and wound infections. *Acinetobacter* is intrinsically resistant to a broad range of antimicrobials. Multi-drug resistant (MDR) *A. baumannii* (defined here as concurrent resistance to imipenem/meropenem, ciprofloxacin, and amikacin) is therefore monitored as infections are more likely to require treatment with polymyxin B or colistin, which are considered last-line antibiotics.

S. aureus is a frequent coloniser of the skin and mucosa. *S. aureus* more commonly causes skin infections but can also spread through the bloodstream and cause a broad range of severe conditions such as pneumonia, endocarditis and osteomyelitis. Methicillin-resistant *S. aureus* (MRSA) are of particular concern due to their resistance to more effective first-line antibiotics used to treat ordinary staphylococcal infections.

Enterococci constitute a part of the normal intestinal microbiota in humans and animals. Most human

enterococci infections are caused by *E. faecalis* and *E. faecium*. Enterococci are intrinsically resistant to many groups of antimicrobials, with severe and penicillin-resistant infections typically treated with vancomycin. *E. faecalis* and *E. faecium* are monitored for resistance to vancomycin. Presence of vancomycin resistance further restricts treatment choice.

Incidence density trends

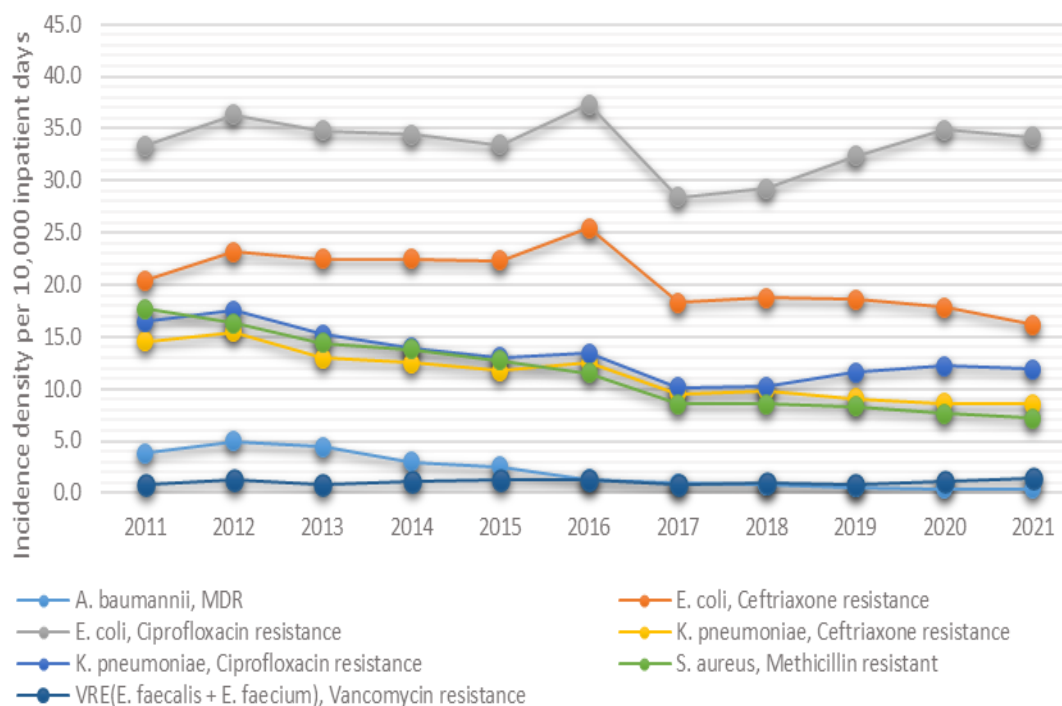
Incidence density is measured as the number of clinical isolates per 10,000 inpatient days. The use of inpatient days as a denominator allows for normalisation across hospitals of different size and patient load.

Public hospital trends

Since 2012, we have observed a decreasing trend in the incidence density of multi-drug resistant (MDR) *A. baumannii*, MRSA and ceftriaxone-resistant *K. pneumoniae* and *E. coli* in public hospitals (Figure 16). These declining rates correspond with the implementation of antimicrobial stewardship programmes in public hospitals in 2011 and are also attributed to the continual enhancement of infection control measures in hospitals.

However, there has been an increase in the incidence of ciprofloxacin-resistant *E. coli* and *K. pneumoniae* since last reported in 2019 (Figure 16), despite a steady decline in ciprofloxacin and overall fluoroquinolone use in public hospitals (Figure 4). VRE rates have also increased since 2019, from 1.1 per 10,000 inpatient days in 2019 to 1.7 in 2021 (Figure 16).

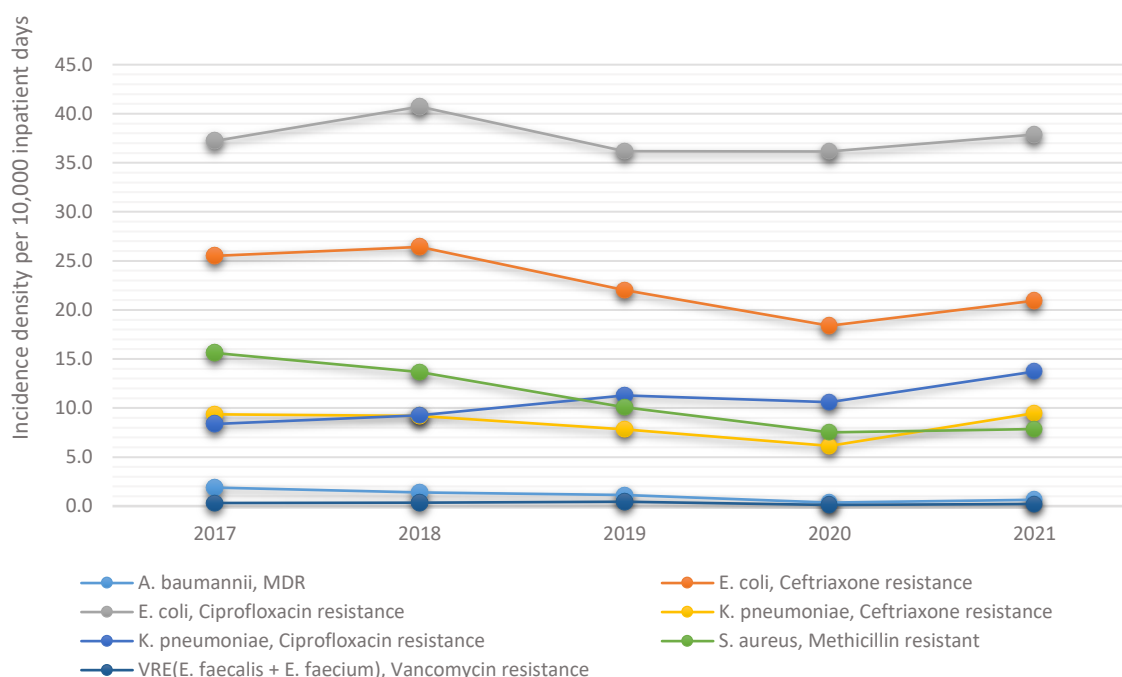
Figure 16. Incidence density of priority AMR organisms in public hospitals, all clinical isolates, 2011 – 2021



Private hospital trends

Decreasing trends of the incidences of MDR *A. baumannii* and MRSA were also observed in private hospitals (Figure 17). Like public hospitals, an increasing trend of ciprofloxacin resistance was observed. The incidences of ceftriaxone-resistant *K. pneumoniae* and *E. coli* rose in 2021 after a decline from 2018 to 2020.

Figure 17. Incidence density of priority AMR organisms in private hospitals, all clinical isolates, 2017 – 2021



Carbapenem-resistant *Enterobacterales* (CRE)

Carbapenem-resistant *Enterobacterales* (CRE), particularly carbapenemase-producing CRE (CP-CRE), are of particular importance due to their resistance to a wide range of antibiotics and the challenges associated with treating patients with CP-CRE infections. This group of pathogens includes the meropenem or imipenem-resistant strains of *E. coli* and *K. pneumoniae*.

The incidence rates of carbapenem-resistant *A. baumannii* has been declining in both public and private hospitals (Figure 18 & 19), with an increase in *P. aeruginosa* incidence in private hospitals in 2021 compared to 2020. The most frequently detected carbapenemases in Singapore (all acute care hospitals) were OXA-type beta-lactamase (OXA), New Delhi metallo-beta-lactamase-mediated carbapenemase (NDM) and *Klebsiella pneumoniae* carbapenemase (KPC; Figure 20).

Figure 18. Trends in incidence density of carbapenem (meropenem or imipenem)-resistant organisms in public hospitals, all clinical isolates, 2011 – 2021

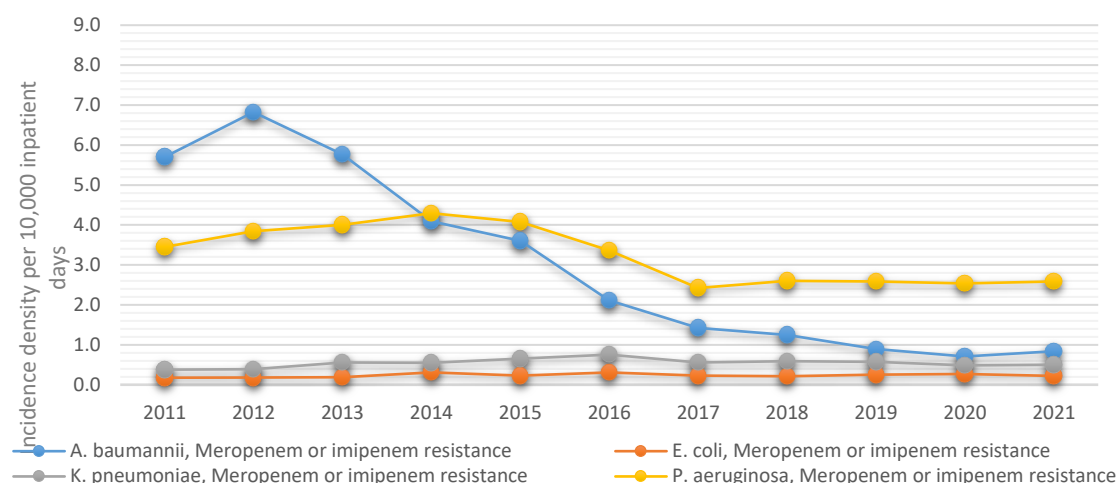


Figure 19. Trends in incidence density of carbapenem (meropenem or imipenem)-resistant organisms in private hospitals, all clinical isolates, 2011 – 2021

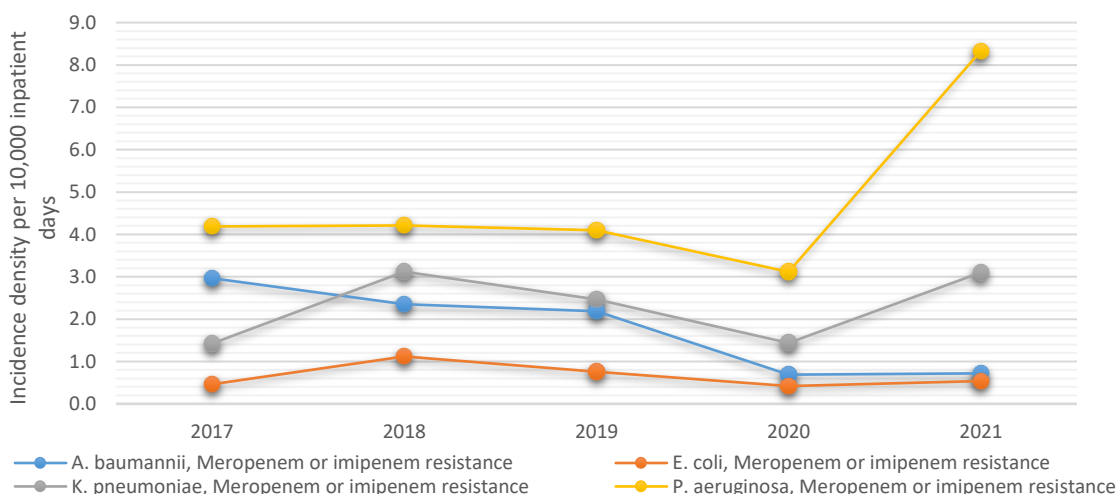
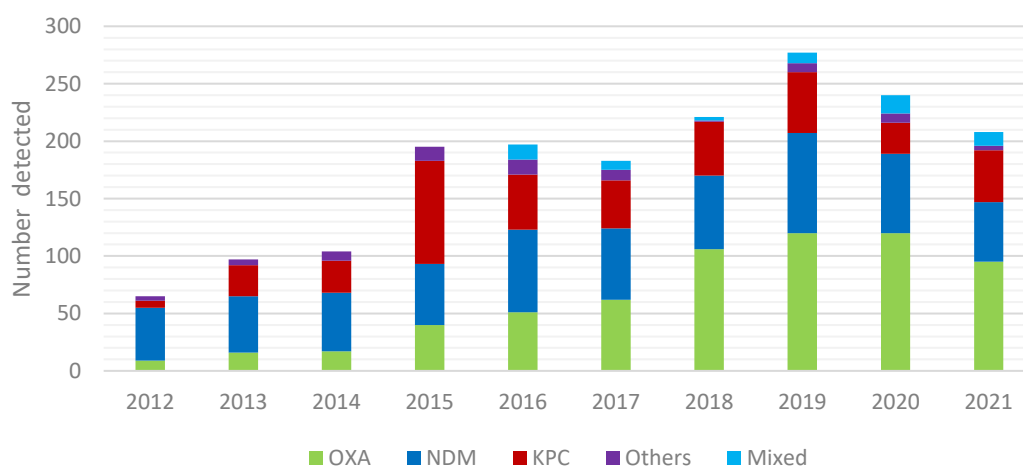


Figure 20. Carbapenemases detected in clinical isolates from all acute care hospitals, 2012 – 2021.



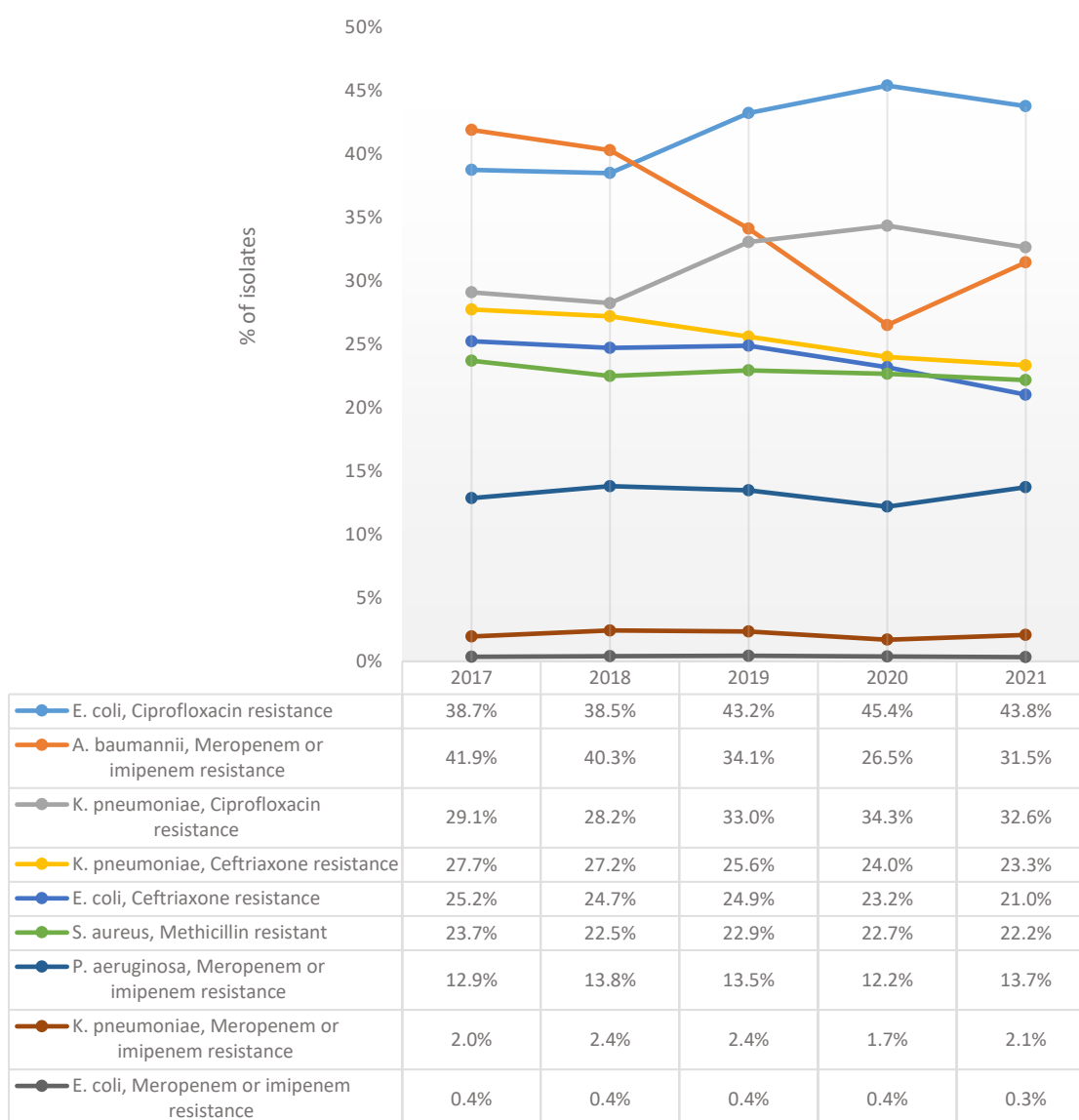
Note: Due to limited data on CPE types in the private sector, data for public and private hospitals are presented together.

Resistance percentage (%R)

The resistance percentage (%R) measures the proportion of isolates that are tested as resistant to a specified antimicrobial, based on CLSI or EUCAST breakpoints. For NARCC's purposes, resistant isolates include those of intermediate susceptibility.

From 2017 to 2021, the average %R of most priority AMR pathogens in acute care hospitals have either decreased or remained stable, except for ciprofloxacin-resistant *E. coli* and *K. pneumoniae*, which were on an increasing trend (Figure 21).

Figure 21. Trends in resistance percentages (%R) of priority pathogen-drug combinations, all clinical isolates from all acute care hospitals, 2017 to 2021



Note: Resistant isolates include those of intermediate susceptibility.

AMR surveillance in primary care

Approximately 20% of the primary healthcare needs are served by 23 polyclinics distributed around the country. In 2019, the AMRCO embarked on systematic and annual collection of data on the resistance profiles (including those of intermediate susceptibility) of *E. coli* and *K. pneumoniae* in urine samples, and *S. aureus* in clinical samples, to better understand resistance levels of key community pathogens.

Overall, average resistance levels remained generally stable from 2019 to 2021. In 2021, *E. coli* displayed the highest resistance to ampicillin (49.2%), ciprofloxacin (33.1%) and co-trimoxazole (25.8%) (Figure 22). *K. pneumoniae* displayed the highest resistance to nitrofurantoin (81.1%) and ciprofloxacin (20.3%) (Figure 23), while 4.8% of *S. aureus* isolates (7 out of 145 isolates) were MRSA (as defined by resistance to cloxacillin or oxacillin; Figure 24). The latter was a decrease from 25.4% in 2020 (15 out of 59 isolates); further monitoring will reveal if this is a downward trend.

Of note, the overall number of carbapenemase producers remained low from 2019 (2 among a total of 706 *K. pneumoniae* and 4894 *E. coli* isolates tested) to 2021 (none among 758 *K. pneumoniae* and 4819 *E. coli* tested). Continued surveillance of resistance rates in the polyclinics is beneficial to provide a proxy measure of community resistance rates over time.

Figure 22. Resistance (%) of *E. coli* from polyclinic urine samples, 2019-2021

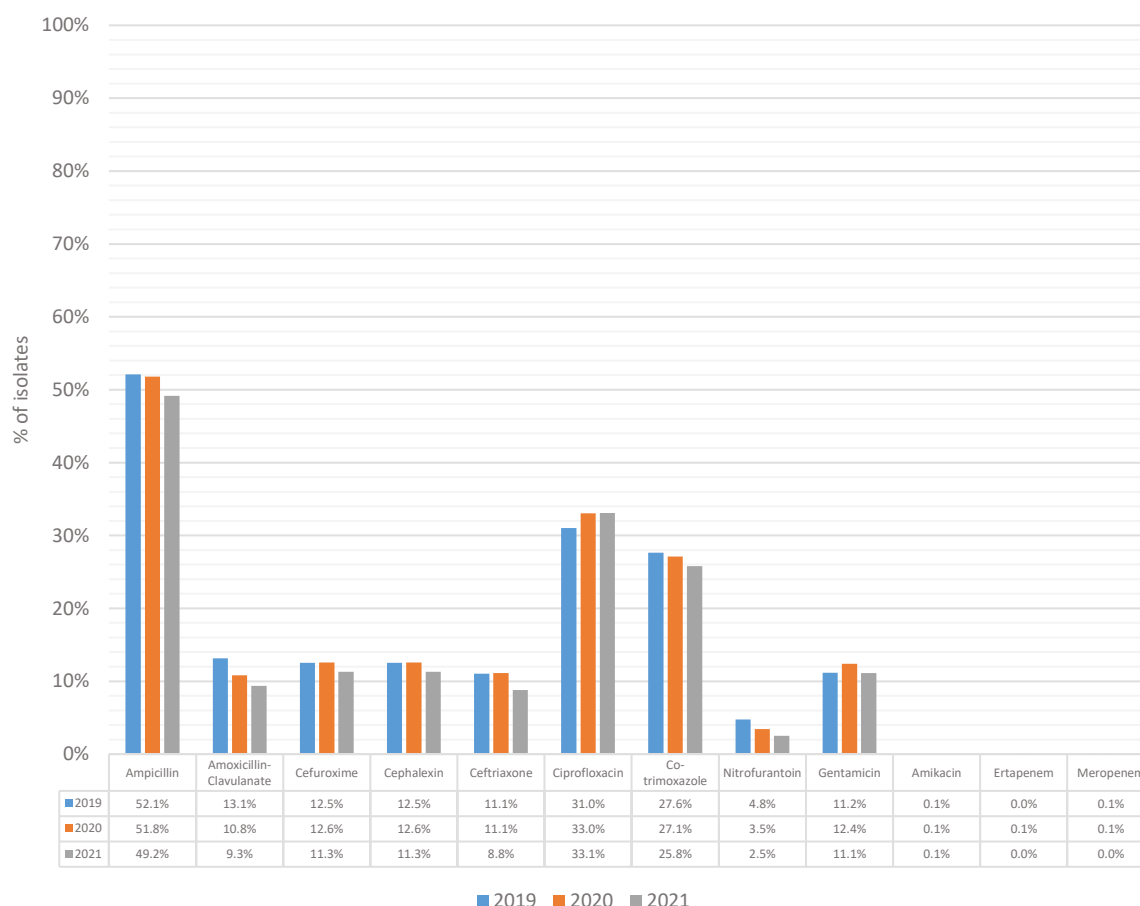
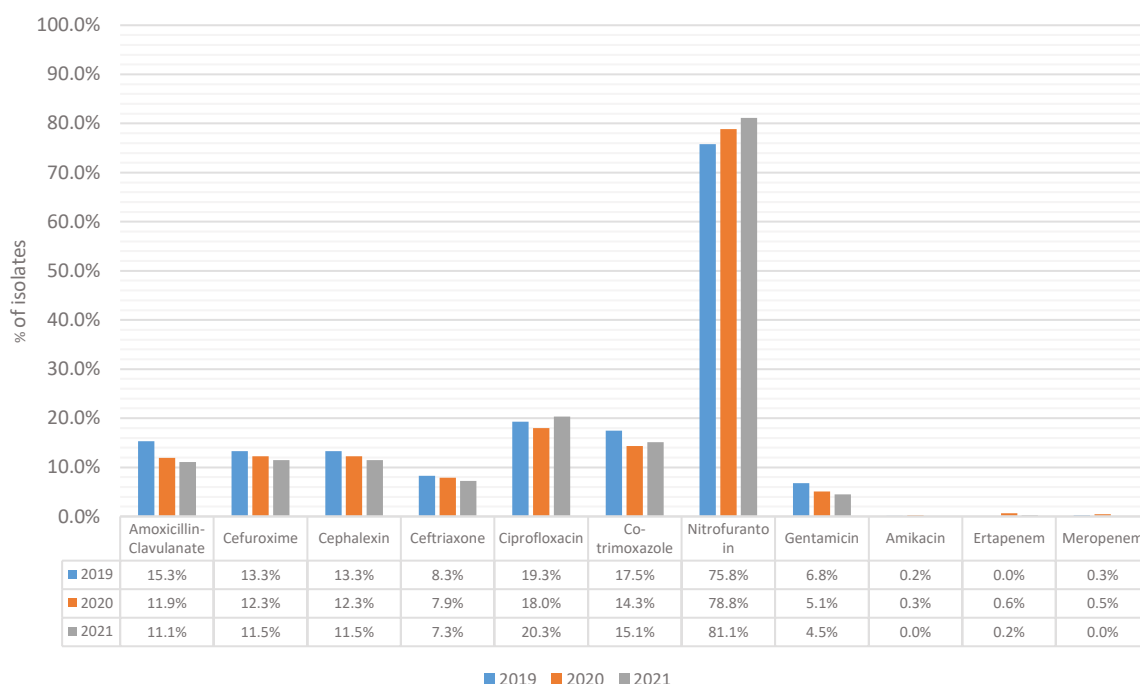
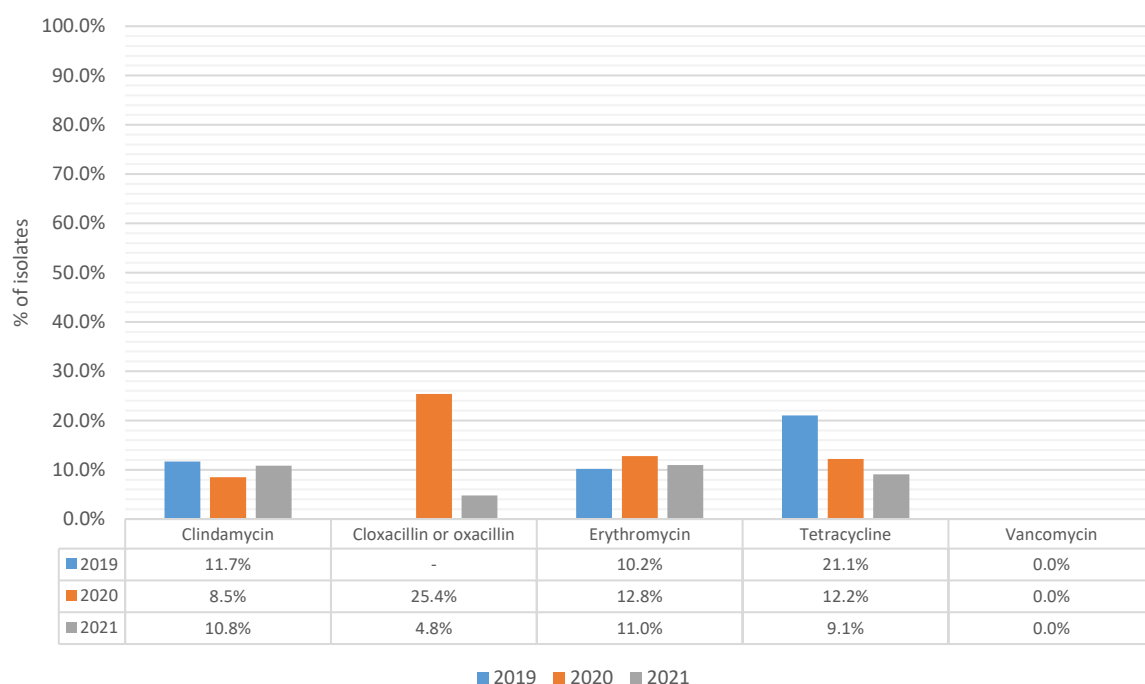


Figure 23. Resistance (%) of *K. pneumoniae* from polyclinic urine samples, 2019-2021

Figure 24. Resistance (%) of *S. aureus* in clinical samples from polyclinics, 2019-2021


Notes: '-' denotes that data was not available for the year indicated. Average %R is the total number of non-susceptible isolates detected across all polyclinics as a percentage of all *S. aureus* isolates tested.

Participation in Global Antimicrobial Resistance Surveillance System (GLASS)

GLASS⁸ was launched by the WHO in 2015 as a collaborative global effort to provide a standardised approach to the collection, sharing and analysis of AMR data. As of 2021, GLASS collected aggregated country data on four priority specimens (blood, stool, urine and genital), and eight organisms (*E. coli*, *K. pneumoniae*, *A. baumannii*, *S. aureus*, *Streptococcus pneumoniae*, *Salmonella* spp., *Shigella* spp., *Neisseria gonorrhoea*), stratified by age group, gender, and origin of infection (hospital vs community). Singapore enrolled in GLASS in September 2019, with the AMRCO and the NPHL appointed by MOH as the national coordinating centre and the AMR reference laboratory, respectively.

Resistance profiles

Aggregate data from three sentinel sites were submitted to GLASS in 2020. These comprised two public acute care hospitals which provided data on blood, urine and stool samples, and one outpatient sexual health clinic providing data on genital samples for gonorrhoea. In 2021, GLASS categorisation of resistant organisms excluded those of intermediate susceptibility.

In 2021, Singapore reported data on bloodstream *E. coli* (n = 1089), *K. pneumoniae* (n = 569), *Acinetobacter* spp (n = 51), *Salmonella* spp (n = 45), *S. pneumoniae* (n = 1) and *S. aureus* (n = 224). Urine isolates were comprised *E. coli* (n= 6232) and *K. pneumoniae* (n = 2082), while stool isolates comprised *Salmonella* spp (n=187) and *Shigella* spp (n= 4). Singapore also reported data for 120 isolates of *N. gonorrhoea* from one outpatient sexual health clinic.

The resistance profiles of GLASS pathogens detected in Singapore's sentinel hospitals are presented in Figures 25 and 26 in terms of resistance percentages (%R). The resistance profile of *N. gonorrhoea* isolates is shown as Figure 27. Only pathogen-drug combinations of at least 10 isolates with AST done are shown in the charts.

The full global and country-level data are available on the GLASS visualisation dashboard at worldhealthorg.shinyapps.io/glass-dashboard/. For details on GLASS methodology and data limitations, please refer to [Global Antimicrobial Resistance and Use Surveillance System \(GLASS\) \(who.int\)](https://www.who.int/glass/en/).

⁸ www.who.int/glass/en/

Figure 25. Resistance (%) of drug-resistant pathogens isolated from (a) blood and (b) urine samples in sentinel hospitals, Singapore 2021

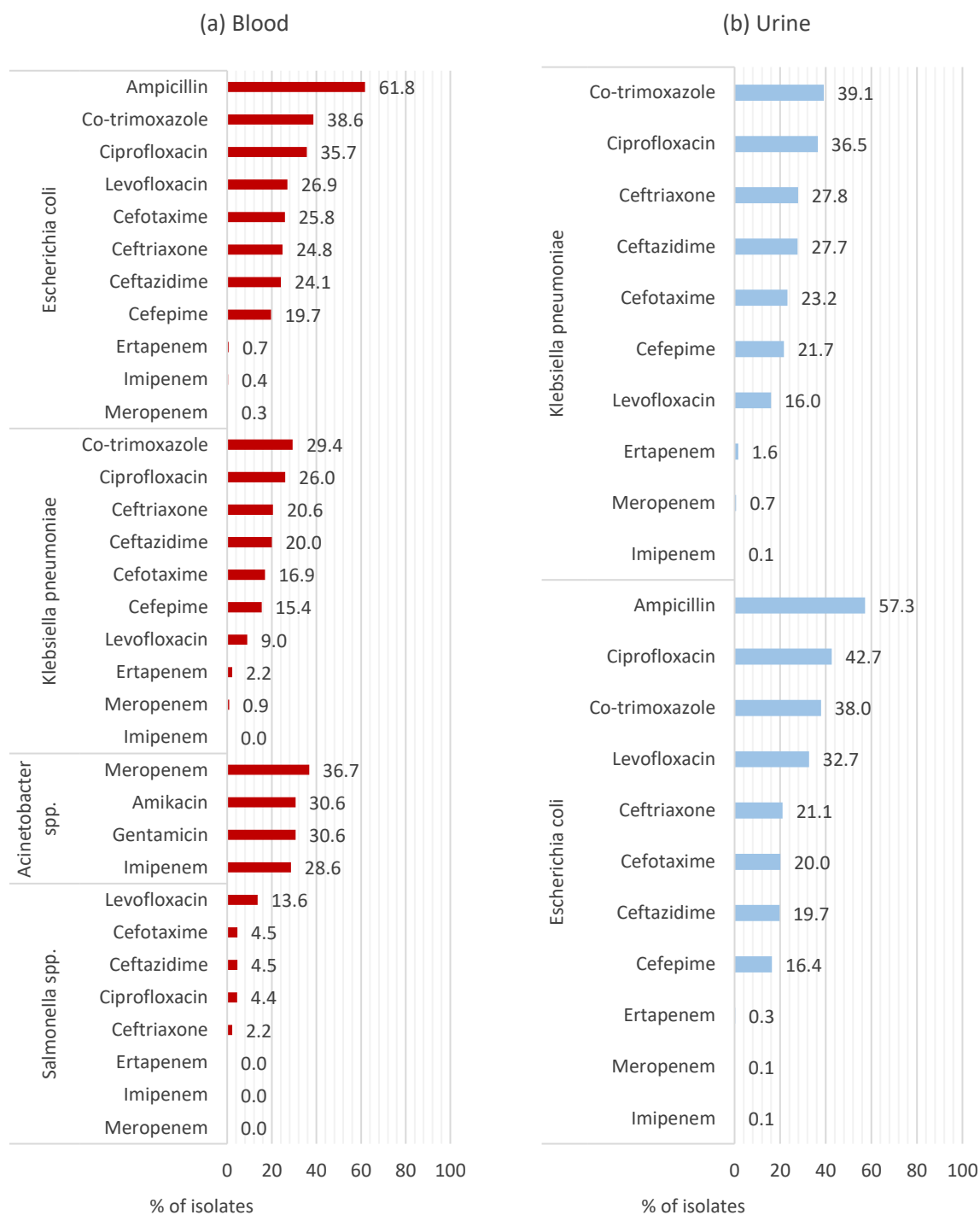
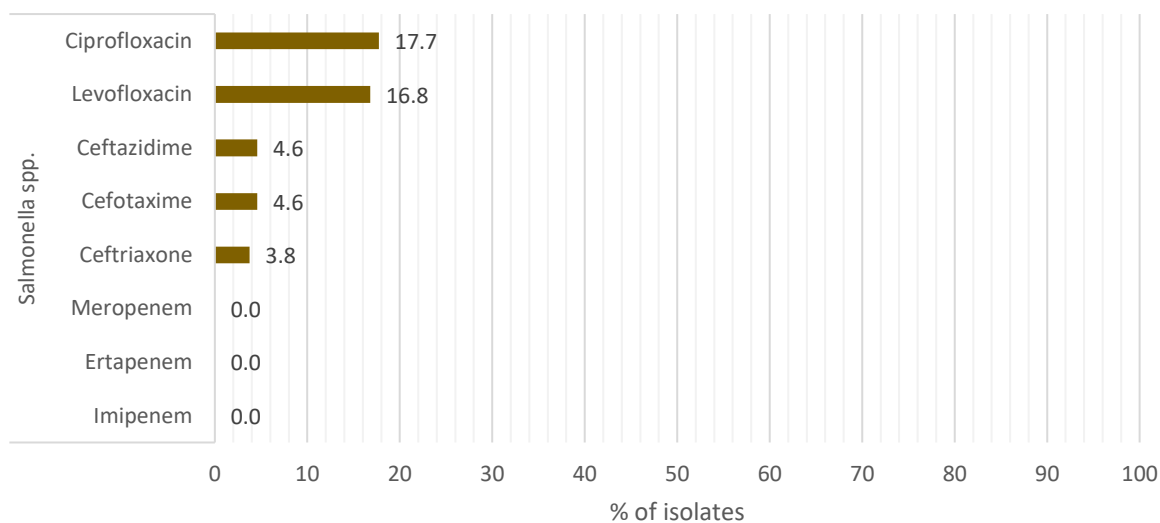
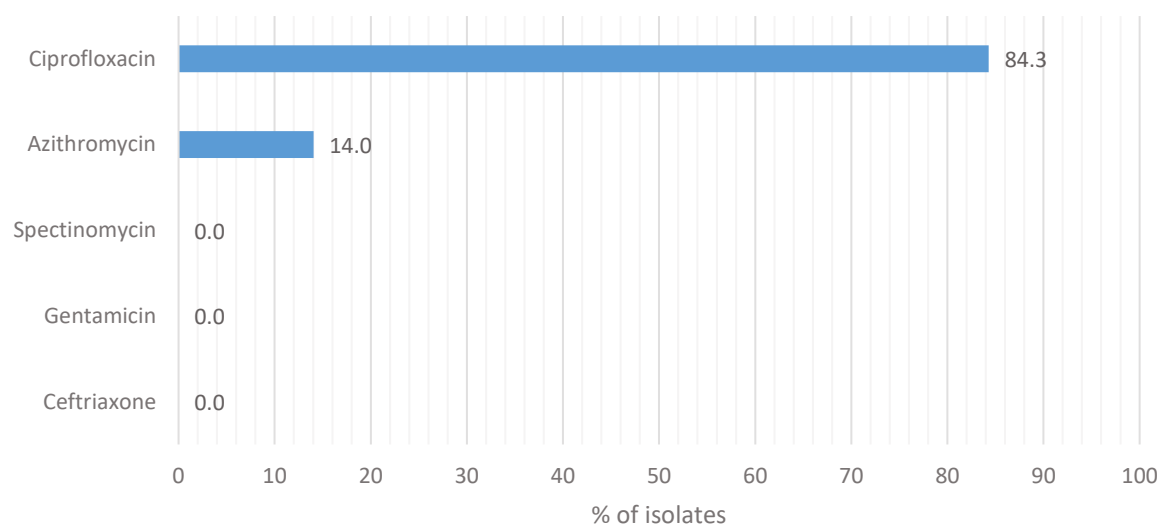


Figure 26. Resistance (%) of drug-resistant *Salmonella* spp isolated from stool samples in sentinel hospitals, Singapore 2021**Figure 27. Resistance (%) of drug-resistant *N. gonorrhoea*, Singapore 2021**

Sustainable Development Goal (SDG) AMR indicators

In 2019, two AMR indicators were introduced for the Sustainable Development Goal (SDG) 3, which is “To ensure healthy lives and promote well-being for all at all ages”.⁹ The AMR indicators measure the reduction in the percentage of bloodstream infections due to selected antimicrobial resistant organisms, specifically, the frequency of bloodstream infection among hospital patients due to (i) *E.*

⁹ <https://sdgs.un.org>

coli resistant¹⁰ to 3rd-generation cephalosporin (e.g., ESBL- *E. coli*) and (ii) MRSA. Methicillin-resistance in *S. aureus* has been calculated by considering AST results for both oxacillin and/or ceftazidime and by taking the maximum resistance value where both antibiotics were tested.

Based on data from Singapore's GLASS sentinel sites and the GLASS dashboard, the proportion of *E. coli* resistant to third-generation cephalosporins in blood samples in 2021 was **25.8%** for Singapore, lower than the global median of 41.0% (IQR 18.18 – 60.0%) of 77 countries, territories or areas (CTAs) reporting to GLASS, but higher than the median of 13.3% (IQR 8.9 – 26.8%) of 35 high income countries (HICs) reporting to GLASS.

The proportion of *S. aureus* resistant to methicillin (MRSA) in 2021 was **32.5%**, comparable to the global median of 32.1% (IQR 9.8 – 45.4%) of 78 CTAs, but higher than the median of 11.0% (IQR 4.3 – 33.1%) of 32 HICs reporting to GLASS.

Caveats:

- (i) Data for these indicators are obtained from only three of 18 acute care hospitals in Singapore and therefore lacks representativeness for the country as a whole. Efforts are on-going to increase the number of sentinel sites to improve the representativeness of Singapore's data.
- (ii) GLASS data records substantial differences in the number of patients that pathogens were isolated from and large variations on country coverage. These impact the quality and relevance of the national antibiotic resistance frequencies. Hence, while GLASS data provide useful estimates for benchmarking, national resistance levels may be non-representative and should therefore be interpreted cautiously.

The GLASS SDGs AMR Indicator dashboard is found at worldhealthorg.shinyapps.io/glass-dashboard/.

¹⁰ Excludes those of intermediate susceptibility

Antimicrobial Resistance in Bacteria in the Food Chain

The national AMR monitoring and surveillance programme for the food chain covers local food-producing animals, animals imported for slaughter, food imports and retail food products. The programme aims to assess the potential impact of AMR in the food chain on consumers and food handlers. The programme covers the testing of common foodborne bacteria, particularly *Salmonella* spp. and *E. coli*, for resistance against clinically and epidemiologically important antimicrobial agents. Efforts are also made to examine the prevalence and AMR profile of ESBL *E. coli* in imported and retail food products.

Salmonella spp. are a major cause of food-borne illness worldwide and in Singapore. Salmonellosis, the infection by non-typhoidal strains of *Salmonella* spp., is a notifiable disease in Singapore. The incidence of Salmonellosis has generally been on a downward trend since 2016, with 1135 cases of salmonellosis reported in 2021 compared with 2214 in 2016¹¹. Out of over 2000 different serovars of *Salmonella enterica*, Enteritidis and Typhimurium are the main serovars associated with non-typhoidal Salmonellosis in Singapore^{12,13}. *Salmonella enterica* serovars are naturally present in the digestive tracts of many animals but are most frequently isolated from poultry and its associated products. *Salmonella* spp. are monitored for specific resistance as well as multi-drug resistance (MDR), defined as resistance to three or more classes of antimicrobials.

E. coli are ubiquitous commensal bacteria found in all warm-blooded animals. *E. coli* in the gut may be exposed to antimicrobials from animal feed and/or water, potentially becoming reservoirs for transferable resistance determinants in the animal or human gut¹⁴. The bacteria also serve as indicators for resistance in different reservoirs along the food chain. As most AMR phenotypes from animal populations are present in commensal bacteria, the effects of AMU and AMR trends are more accurately reflected in commensal bacteria than in food-borne pathogens¹⁵. Most strains of *E. coli* are non-pathogenic but have the potential to transfer resistance determinants to pathogenic Gram-negative bacteria. Hence, *E. coli* are monitored for specific resistance as well as MDR. ESBL *E. coli* are of specific concern due to their concurrent resistance to many other antibiotics.

Key findings from surveillance of these organisms in the food supply chain are summarised below.

¹¹ Ministry of Health, Singapore. Weekly Infectious Diseases Bulletin. [Online] <https://www-moh-gov-sg-admin.cwp.sg/resources-statistics/infectious-disease-statistics>

¹² Salmonellosis, non-typhoidal. In: Ooi PL, Boudville I, Chan M, Tee N, eds. Communicable diseases control. Singapore: S-FETP, 2020; 264-266.

¹³ Aung, K.T., et al. Characterisation of *Salmonella* Enteritidis ST11 and ST1925 Associated with Human Intestinal and Extra-Intestinal Infections in Singapore. Int. J. Environ. Res. Public Health. 2022, Vol. 19, p. 5671.

¹⁴ Food and Agriculture Organisation of the United Nations (FAO), 2019. *Regional Antimicrobial Resistance Monitoring and Surveillance Guidelines Volume 1 (Monitoring and surveillance of antimicrobial resistance in bacteria from healthy food animals intended for consumption)*.

¹⁵ European Food Safety Authority (EFSA), 2018. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2016. *EFSA Journal*, 16(2), 5182.

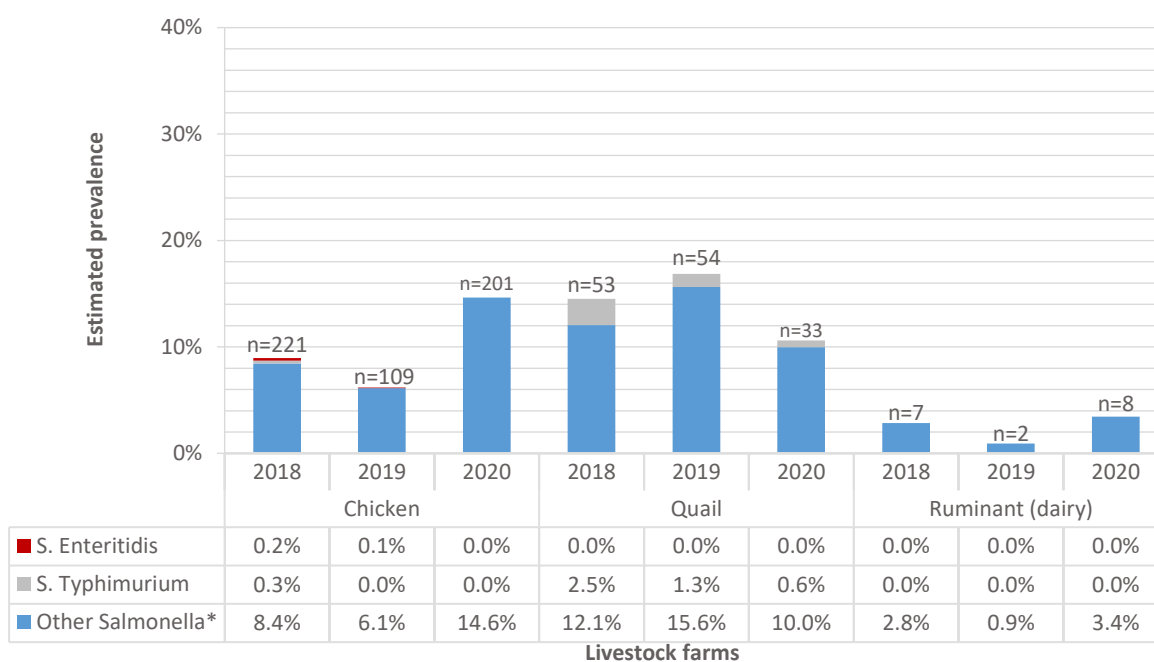
Antimicrobial resistance in *Salmonella*

In Production animals

The prevalence and AMR profiles of *Salmonella* in local chicken and quail farms have been under monitoring since 2008. This surveillance programme was administered by NParks from 2008 to June 2021, with SFA assuming its administration from July 2021. This Report covers data on *Salmonella* isolated from local poultry farm up to 2020; data from the transition period of 2021 will be included in the next One Health AMR surveillance report. Surveillance of the ruminant (dairy goat and cattle) farms was initiated in November 2017 and remains under the purview of NParks.

The prevalence of *Salmonella* Enteritidis and *Salmonella* Typhimurium on local farms continue to remain low (Figure 28). *Salmonella* Enteritidis was not isolated in local poultry and ruminant farms in 2020. Two (0.6%) *S. Typhimurium* were isolated in local quail farms in 2020. *Salmonella* Typhimurium was not isolated in the other farms in 2020. The overall prevalence of *Salmonella* spp., mostly Group C and E *Salmonella* spp., were 3.4 - 14.6% in 2020.

Figure 28. Estimated overall prevalence of *Salmonella* spp. in local farms, 2018 –2020 (n = number of samples with *Salmonella* spp. isolated)

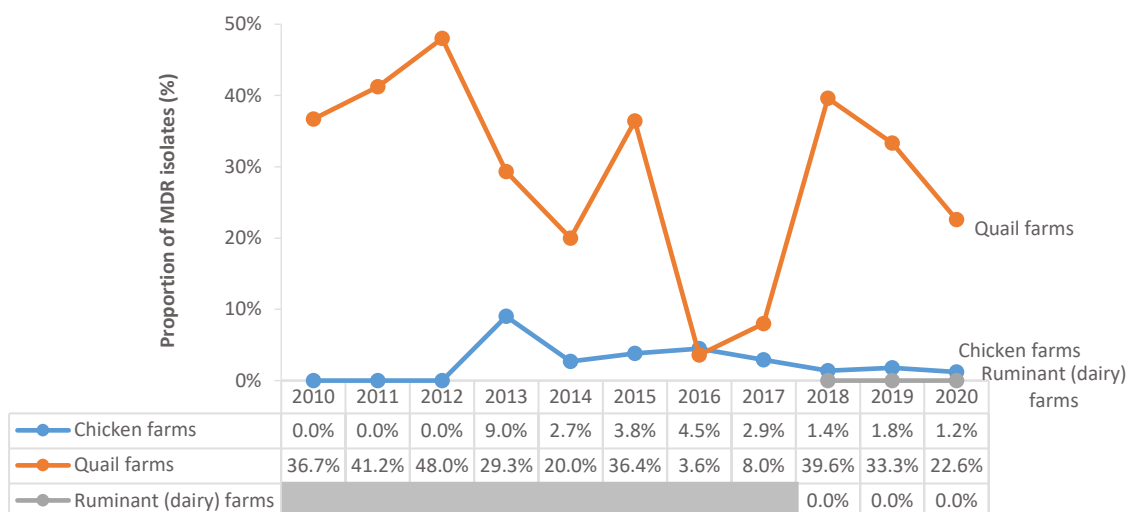


MDR *Salmonella* in terrestrial farm animals

MDR *Salmonella* is a worldwide challenge, particularly when occurring in serovars of public health importance. Farm isolates were tested for susceptibility to antibiotics by broth microdilution, applying CLSI M100 and/or VET01 standards and interpretative breakpoints as appropriate. Since 2020, the Sensititre™ Asia Surveillance Plates for *Salmonella*/*E. coli* (ASSECAF and ASSECB; Appendix 2) have been used for monitoring resistance for *Salmonella* spp. and *E. coli*, which allow AST methods and antibiotic panels to be harmonised with other ASEAN regional laboratories to enable comparable analyses of AMR in food animals in the region.

The proportion of MDR *Salmonella* spp. was higher in quail farms (22.6% in 2020) than in other local terrestrial farms; however, none of the MDR isolates was *S. Enteritidis* and *S. Typhimurium*. The proportion of MDR *Salmonella* spp. isolated from chicken farm samples was 1.2% in 2020 (Figure 29). No MDR *Salmonella* spp. was isolated in ruminant farms from 2018 to 2020.

Figure 29. Proportion of MDR isolates among *Salmonella* spp. from the local terrestrial farms, 2010 – 2020.



Note: AMR surveillance in ruminant farms started in 2018; AST data prior to 2018 are not available for ruminant farms. AST were performed with 11 antimicrobials in 2018–2021, as compared to 4 antimicrobials (ampicillin, chloramphenicol, streptomycin and tetracycline) from 2010 to 2017.

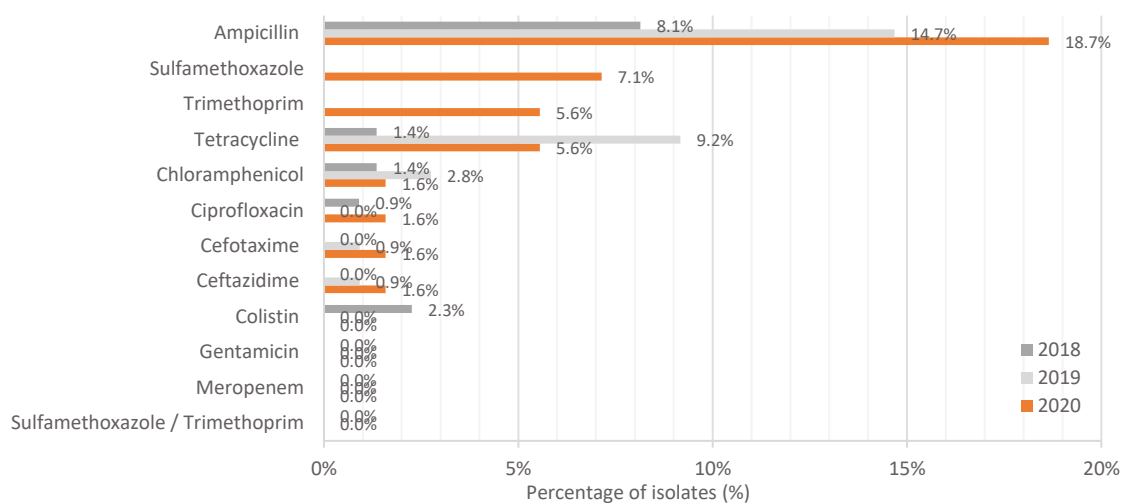
Resistance profiles of Salmonella from local farms

Ruminant farms

Salmonella spp were infrequently isolated from ruminant farm samples, with typically less than 4% recovered in the samples tested. None were serovars Enteritidis or Typhimurium. *Salmonella* isolated from local ruminant farms in 2020 demonstrated susceptibility to all antibiotics tested, including ampicillin, ceftazidime, cefotaxime, chloramphenicol, ciprofloxacin, colistin, gentamicin, meropenem, sulfamethoxazole, tetracycline, and trimethoprim.

Chicken layer farms

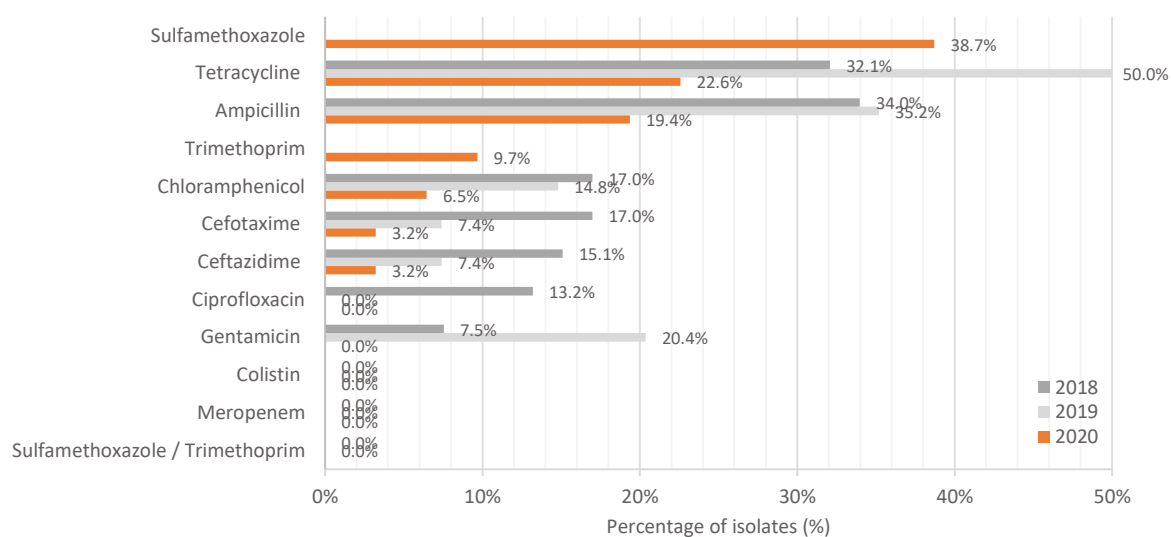
Salmonella isolates (n = 252) from chicken farms in 2020 were most frequently resistant to ampicillin (18.7%), an increase from 14.7% in 2019 (Figure 30). The percentage of isolates resistant to ciprofloxacin and third-generation cephalosporin also increased slightly (1.6% in 2020 compared with 0.0% in 2019). While all *Salmonella* isolates from chicken farms had been previously susceptible to sulfamethoxazole/trimethoprim when tested in combination, 7.1% and 5.6% of *Salmonella* isolates in 2020 exhibited resistance to sulfamethoxazole and trimethoprim respectively, when tested separately. However, resistance to tetracycline and chloramphenicol decreased in 2020 compared with 2019. All *Salmonella* spp. isolated from local chicken farms in 2020 remained susceptible to colistin, gentamicin, and meropenem (Figure 30).

Figure 30. Percentage resistance of *Salmonella* spp. isolated from local chicken farms, 2018-2020

Note: Starting in 2020, the monitoring of resistance uses the Sensititre™ Asia Surveillance Plates (ASSECAF and ASSECB; see Appendix 2), which monitors trimethoprim and sulfamethoxazole resistance separately.

Quail layer farms

Salmonella isolates in 2020 demonstrated decreased percentage resistance to most antimicrobials tested compared to isolates in 2018 and 2019 (Figure 31). In addition, all *Salmonella* spp. isolated from local quail farms in 2020 demonstrated susceptibility to ciprofloxacin, colistin, gentamicin, and meropenem (Figure 31). Previously, all *Salmonella* isolated from quail farms in 2018 and 2019 were susceptible to sulfamethoxazole/trimethoprim when tested in combination. When tested separately, 38.7% and 9.7% of the *Salmonella* isolated in 2020 were resistant to sulfamethoxazole and trimethoprim, respectively.

Figure 31. Percentage resistance of *Salmonella* spp. isolated from local quail layer farms, 2018-2020

Note: Starting in 2020, the monitoring of resistance uses the Sensititre™ Asia Surveillance Plates (ASSECAF and ASSECB; see Appendix 2), which monitors trimethoprim and sulfamethoxazole resistance separately.

Discussion

We observed that poultry farm *Salmonella* isolates exhibited higher resistance percentages to antimicrobials of the class of folate pathway antagonists, such as sulfamethoxazole and trimethoprim, when these antimicrobials were tested separately than when tested in combination. The practice of monitoring using sulfamethoxazole/trimethoprim in combination may therefore have previously underestimated the resistance to this class of antimicrobials. In veterinary medicine, sulfamethoxazole and trimethoprim are commonly prescribed to treat bacterial infections in terrestrial farms, although the overall antimicrobial usage in terrestrial livestock is generally low. It is noteworthy that annual sales of sulphonamides, including trimethoprim, has remained low in the animal sector (Figure 14).

These observed changes are unlikely to have significant impact on public health: Across all local farms, MDR *S. Enteritidis* and MDR *S. Typhimurium* were not detected in 2020. Other serovars detected were those less frequently associated with human cases of salmonellosis. Nevertheless, these findings underscore the importance for continual monitoring of antimicrobial-resistant foodborne pathogens on local farms.

In Imported and Retail Food Products

Salmonella in Imported food

Detection rates of Salmonella in imported raw meat products.

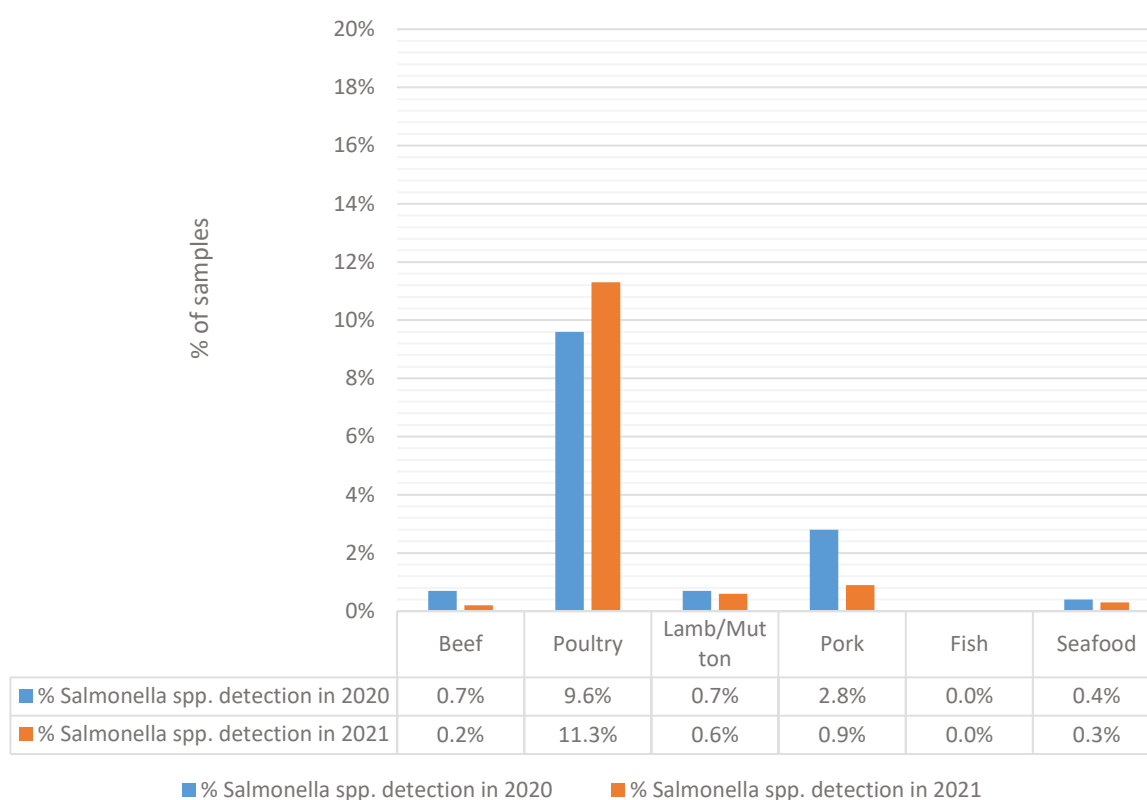
Under SFA's import control surveillance programme, 479 *Salmonella* isolates were obtained from 8470 (5.7%) imported raw meat products (beef, chicken mutton/lamb, pork, fish and other seafood) tested in 2020 and 2021. The numbers of samples tested in 2020 and 2021 are shown in Table 2.

Table 2. Number of imported food product samples tested in 2020 and 2021 for *Salmonella* spp.

	Beef	Poultry	Lamb/Mutton	Pork	Fish	Seafood	Total samples tested
2020	1184	1132	427	834	132	279	3988
2021	1372	1272	354	964	136	384	4482

The prevalence of *Salmonella* spp. was relatively higher in raw poultry and pork products as compared to other meats, with poultry being the main contributing source (Figure 32).

The average detection rate of *Salmonella* in poultry and pork in 2020-2021 (10.6%, 430 isolates from 4202 samples) was higher than that for 2018-2019¹⁷ (6.7%; 488 isolates from 7296 samples). Of 430 *Salmonella* isolates from poultry and pork in 2020-2021, 74.0% (318/430) were obtained from chicken meat products.

Figure 32: Detection of *Salmonella* spp. in imported food products and establishments (2020-2021)

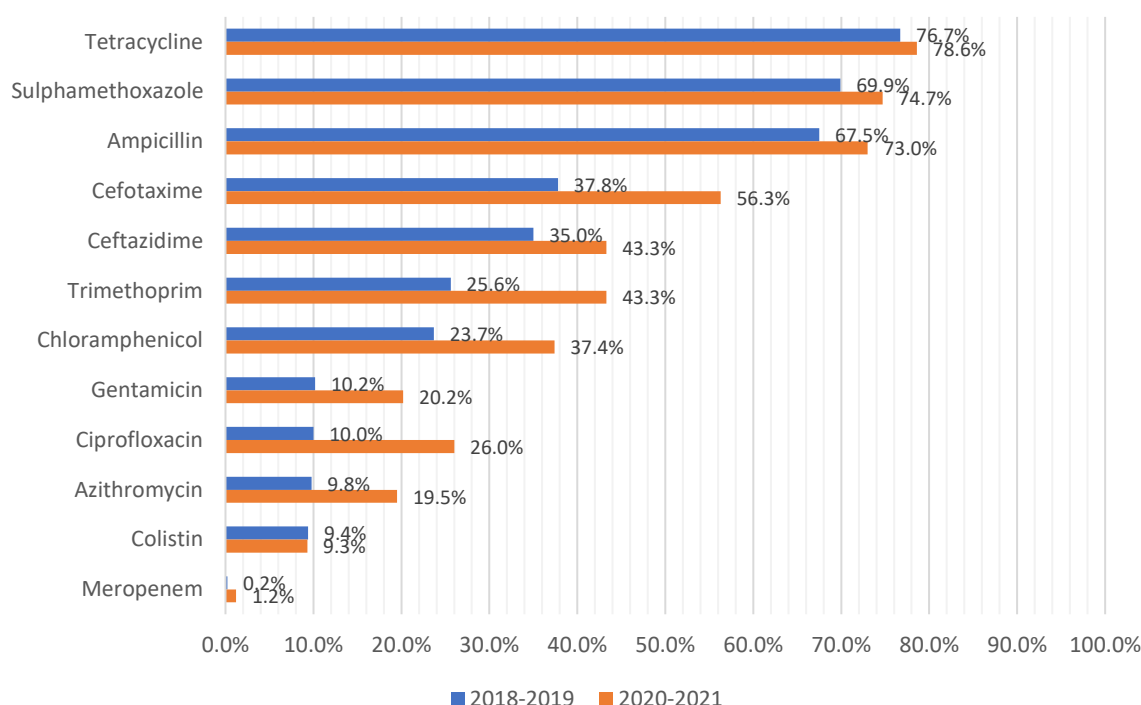
Resistance profile of *Salmonella* isolates from imported food products

Salmonella isolates were subjected to AST by microbroth dilution applying CLSI breakpoints (M100, 30th Edition). Tests for Minimum Inhibitory Concentration (MIC) were performed with 10 classes of 11 antimicrobials (ampicillin, cefotaxime, ceftazidime, ciprofloxacin, chloramphenicol, colistin, gentamicin, meropenem, sulfamethoxazole, tetracycline and trimethoprim) using the Sensititre™ Asia Surveillance Plates for *Salmonella*/*E. coli* (see Appendix II. AMR Methodology). *Salmonella* spp. serovars was determined by serotyping and/or Whole Genome Sequencing (WGS).

*Resistance profiles of *Salmonella* isolates in poultry and pork*

Figure 33 shows the resistance profiles of *Salmonella* isolated from imported poultry and pork products from 2018 to 2021, with the number of isolates shown in Table 3.

Over 70% of *Salmonella* isolates tested were resistant to tetracycline, sulfamethoxazole and ampicillin. Of the isolates tested, 43.3% to 56.3% were resistant to cephalosporins such as cefotaxime and ceftazidime. Compared to 2018-2019¹⁷, there was an increase in resistance percentages to most antibiotics tested.

Figure 33. Resistance (%) of *Salmonella* isolates from imported poultry (chicken, duck) and pork meat, 2018 - 2021**Table 3. Number of *Salmonella* isolates detected in imported raw poultry and pork, 2018-2021**

	<u>Chicken</u>	<u>Duck</u>	<u>Pork</u>	<u>Total</u>
2018 - 2019	311	61	117	489
2020 - 2021	318	46	66	430

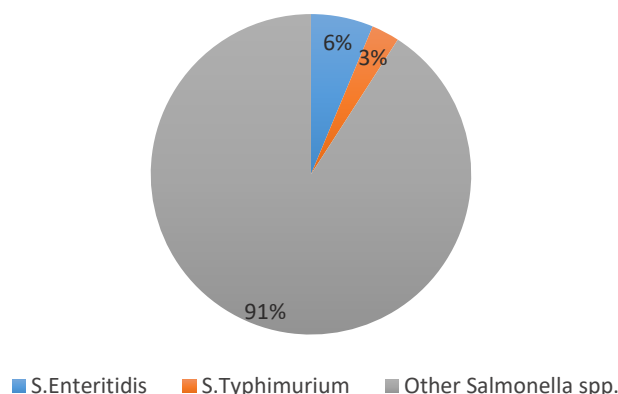
MDR Salmonella in chilled and frozen chicken

Among 318 *Salmonella* isolates from imported chilled and frozen chicken meat products in 2020 and 2021, 77.3% of the isolates tested were found to be MDR (Table 4). Isolates from frozen chicken products were generally more likely to be MDR than isolates from chilled chicken, except in 2021, where the percentage of MDR isolates in both chilled and frozen chicken were similar. *Salmonella* Enteritidis and Typhimurium serovars accounted for 6% and 3% of isolates in 2020-2021 respectively; the majority were of other serovars (Figure 34).

Table 4. Percentage of MDR *Salmonella* in imported chicken meat products, 2018 – 2021

<i>Product type</i>	2018	2019	2020	2021
Imported chilled chicken meat products	67.8% (40/59)	73.2% (41/56)	55% (22/40)	89.0% (73/82)
Imported frozen chicken meat products	74.1% (80/108)	79.5% (70/88)	64.6% (64/99)	89.7% (87/97)

Note: MIC tests using Sensititre™ Asia Surveillance Plates for *Salmonella*/*E. coli* were introduced in 2018. Due to changes in methodology, 2018-2021 data are not trended with MDR *Salmonella* data reported for 2011-2017¹.

Figure 34. Distribution of *Salmonella* serovars detected in imported chicken products, 2020 – 2021 (n = 318)

Salmonella from retail food products

Under the market monitoring programme (MMP), SFA monitors cooked/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). SFA also monitors raw food such as poultry, meat, vegetables and seafood products from wet markets and supermarkets under its MMP.

Overall, the detection of *Salmonella* in raw meat products remained relatively stable from import (5.7%) and retail (4.8%) sectors in 2020 and 2021 (Figure 35), which were similar to rates for 2018 and 2019¹⁷ (Table 5). Of 119 *Salmonella* isolates detected in retail chicken products, 9% were Enteritidis and 6% were Typhimurium (Figure 36).

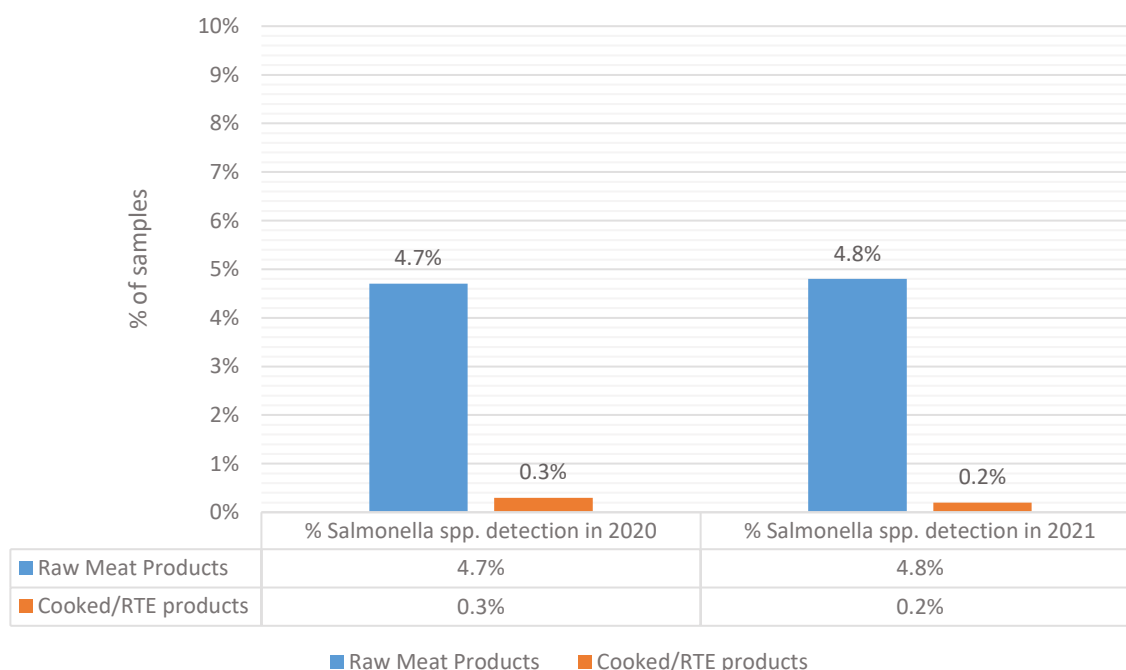
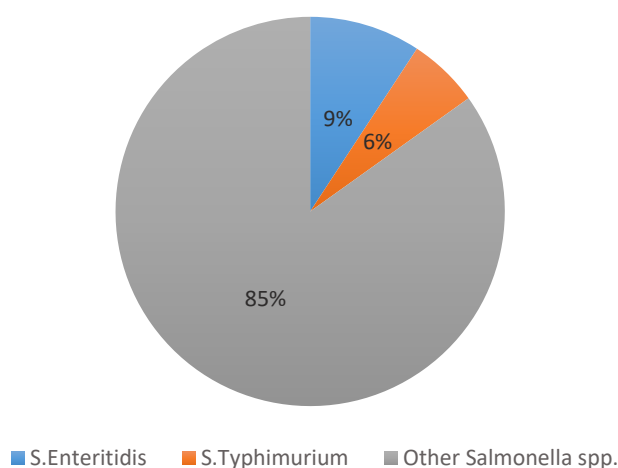
Figure 35. Detection of *Salmonella* in Retail and Cooked/RTE food (2020 – 2021)

Table 5. Number of raw meat and cooked/RTE products tested for *Salmonella* spp in 2020-2021

	Raw Meat Products	Cooked/RTE products
2020	1204	1658
2021	3021	4547
2-year Total	4225	6245
No. of isolates obtained	202	15
Detection rate	4.8%	0.2%

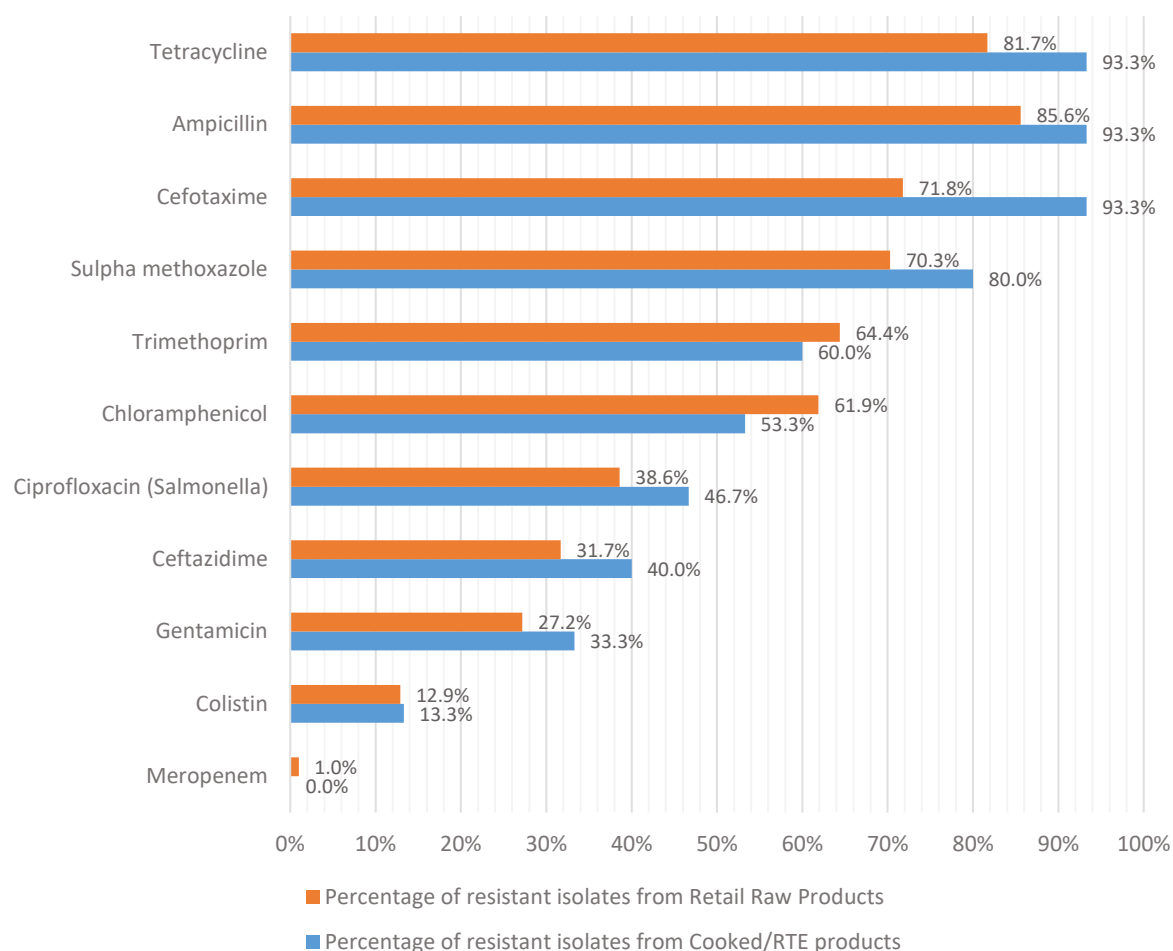
Figure 36. Distribution of *Salmonella* serovars detected in retail chicken products, 2020-2021 (n=119)

Resistance profiles of Salmonella from retail food

The antibiotic resistance profiles of a total of 217 *Salmonella* isolated from retail food in 2020 and 2021 were examined, comprising 202 isolates from raw meat and 15 isolates from cooked/RTE products (Table 5). There was an overall increase in the resistance rates of isolates to most antibiotics tested compared to the period from 2017-2018¹⁷.

Of the isolates tested, over 80% of the isolates were resistant to tetracycline and ampicillin (Figure 37). In comparison, approximately 60% and 50% of isolates were resistant to tetracycline and ampicillin, respectively, in 2017-2018. In addition, more than 60% of isolates were resistant to trimethoprim, sulfamethoxazole, chloramphenicol and to cephalosporins such as cefotaxime. Compared to data in the 2019 report, the percentage of isolates resistant to cefotaxime increased from approximately 12% in 2017-2018 to 93% in 2021. Likewise, resistance to ciprofloxacin increased from approximately 1% to 46.7% in 2021.

Salmonella from retail chilled meat products, 51.4% and 9.9% were found to be MDR in 2020 and 2021 respectively. MDR *Salmonella* were also isolated from frozen products, but numbers were too few to provide any reliable estimates of MDR rates (Table 6).

Figure 37. Percentage resistance of *Salmonella* isolates from retail raw meat (n = 202) and cooked /RTE products (n=15), 2020 - 2021**Table 6. Percentage of MDR *Salmonella* in retail chicken meat products, 2020 - 2021**

<i>Product type</i>	2017-2018 ¹⁶	2020	2021
Retail chilled chicken meat products	50.9% (108/204)	51.4% (19/37)	9.9% (8/81)
Retail frozen chicken meat products		100% (4/4) [^]	66.7% (4/6) [^]

Note: MIC tests using Sensititre™ Asia Surveillance Plates for *Salmonella*/*E. coli* were introduced in 2018. Due to changes in methodology, 2020-2021 data are not trended with MDR *Salmonella* data reported for 2011-2019.

[^] Number of isolates recovered were below the threshold of reliability.

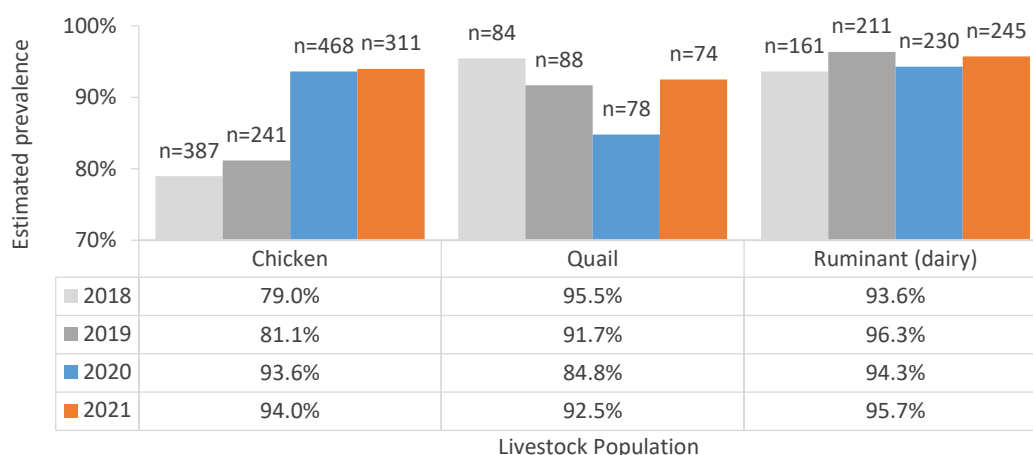
¹⁶ One Health Report on Antimicrobial Utilisation and Resistance, 2019.

Antimicrobial resistance in *E. coli*

In Production animals

Monitoring of AMR profiles of indicator *E. coli* from local poultry and ruminant farms was introduced in November 2017. *E. coli* was isolated from 776 (92.8%) and 630 (94.5%) healthy local farm animals in 2020 and 2021 respectively. As expected of commensals, the prevalence of *E. coli* in local poultry and ruminant farms ranged from 84.8 – 94.3% in 2020 to 92.5 – 95.7% in 2021.

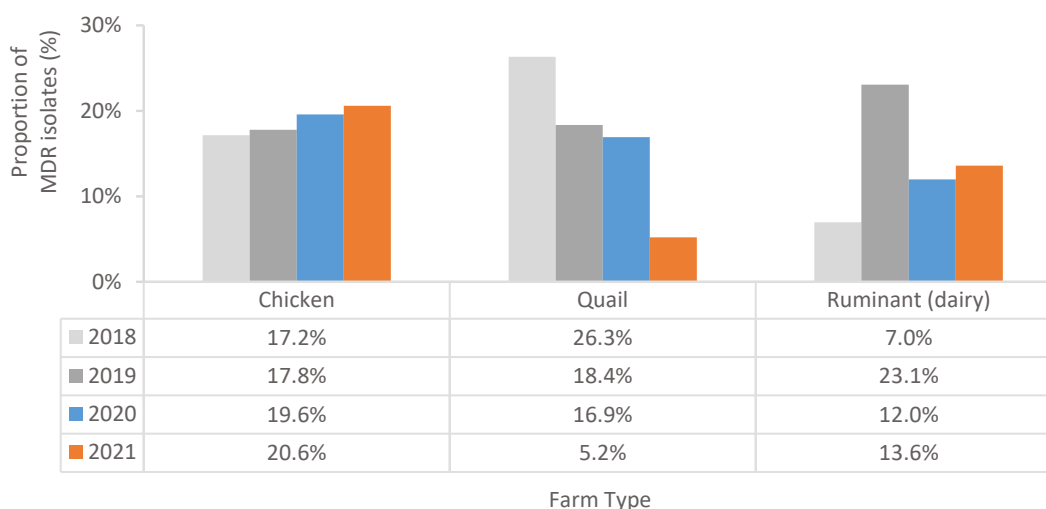
Figure 38. Estimated prevalence of *E. coli* in local livestock populations, 2018-2021 (n = number of samples with *E. coli* isolated)



MDR *E. coli*

From 2018 to 2021, there was an increasing trend in the proportion of MDR *E. coli* isolated from local chicken farms, whereas a decreasing trend was observed for MDR *E. coli* from local quail farms. For ruminant farm isolates, the proportion of MDR *E. coli* decreased from 23.1% in 2019 to 12.0% and 13.6% in 2020 and 2021, respectively.

Figure 39. Proportion of MDR *E. coli* isolated in local farms, 2018 - 2021

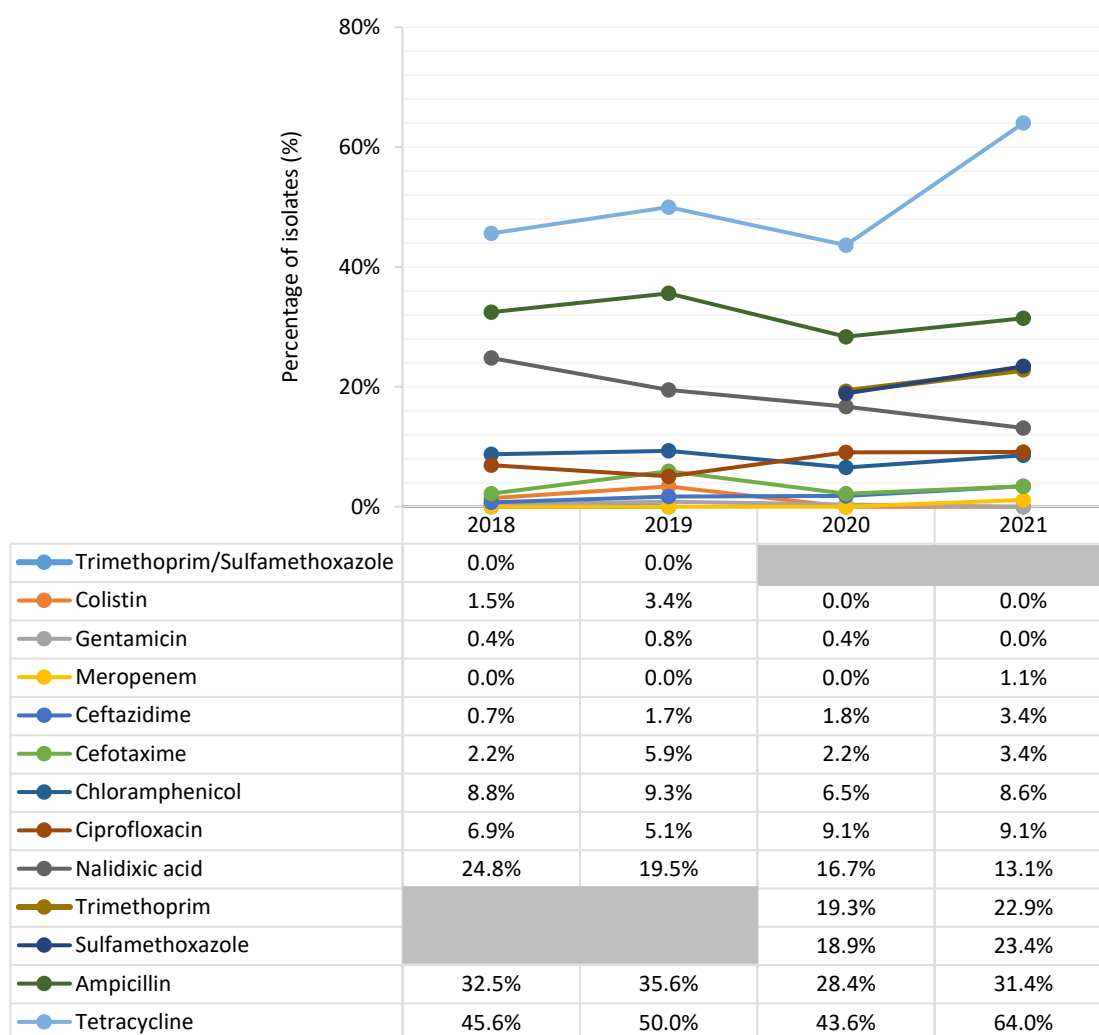


*Resistance profiles in Indicator E. coli*Chicken farms

From 2018 to 2021, percentage resistances for most antimicrobials tested were relatively stable, with a steady decreasing trend in resistance to nalidixic acid, and a more gradual increase in ciprofloxacin resistance since 2019. Resistance to ceftazidime also appears to be on a gradual upward trend since 2018. Tetracycline and ampicillin resistance proportions remain the highest of the antibiotics tested, with a marked increase in tetracycline resistance from 2020 to 2021.

In 2020, *E. coli* isolates (n=275) were most frequently resistant to tetracycline, followed by ampicillin, trimethoprim and sulfamethoxazole, while being fully susceptible to colistin and meropenem. In 2021, isolates (n=175) were most frequently resistant to tetracycline, followed by ampicillin, sulfamethoxazole and trimethoprim. All isolates were susceptible to colistin and gentamicin.

Figure 40. Percentage resistance of *E. coli* isolated in local chicken farms, 2018-2021



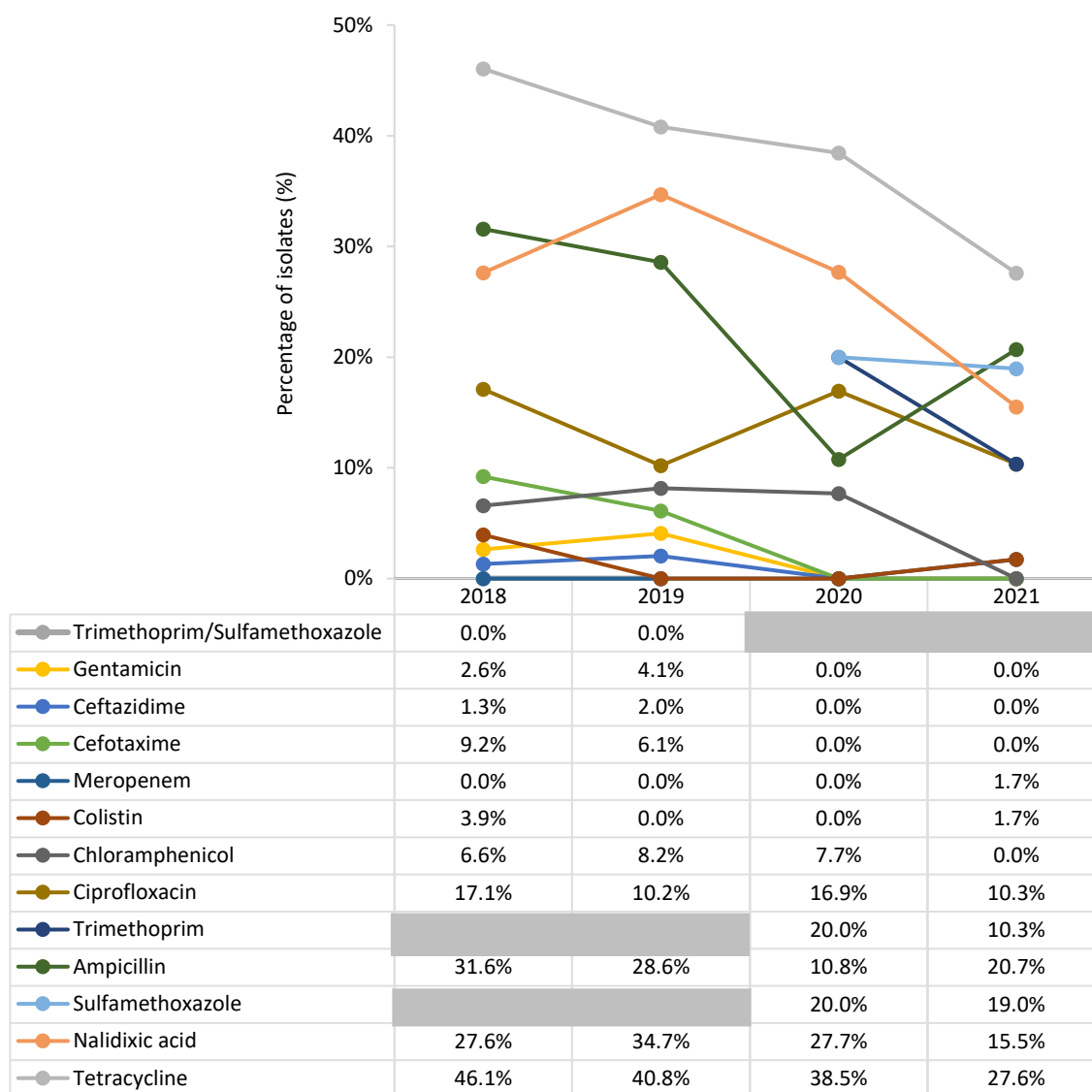
Note: Starting in 2020, the monitoring of resistance uses the Sensititre™ Asia Surveillance Plates (ASSECAF and ASSECB; see Appendix 2), which monitors trimethoprim and sulfamethoxazole resistance separately.

Quail farms

Since 2018, there has been a declining trend in resistance proportions to several antibiotics, including tetracycline and nalidixic acid (Figure 41).

In 2020, *E. coli* isolates (n=65) from quail farms were most frequently resistant to tetracycline, followed by nalidixic acid, sulfamethoxazole and trimethoprim, while all were susceptible to cefotaxime, ceftazidime, colistin, gentamicin and meropenem. In 2021, isolates (n=58) were most frequently resistant to tetracycline, followed by ampicillin and sulfamethoxazole and susceptible to cefotaxime, ceftazidime, chloramphenicol and gentamicin.

Figure 41. Percentage resistance of *E. coli* isolated in local quail farms, 2018-2021

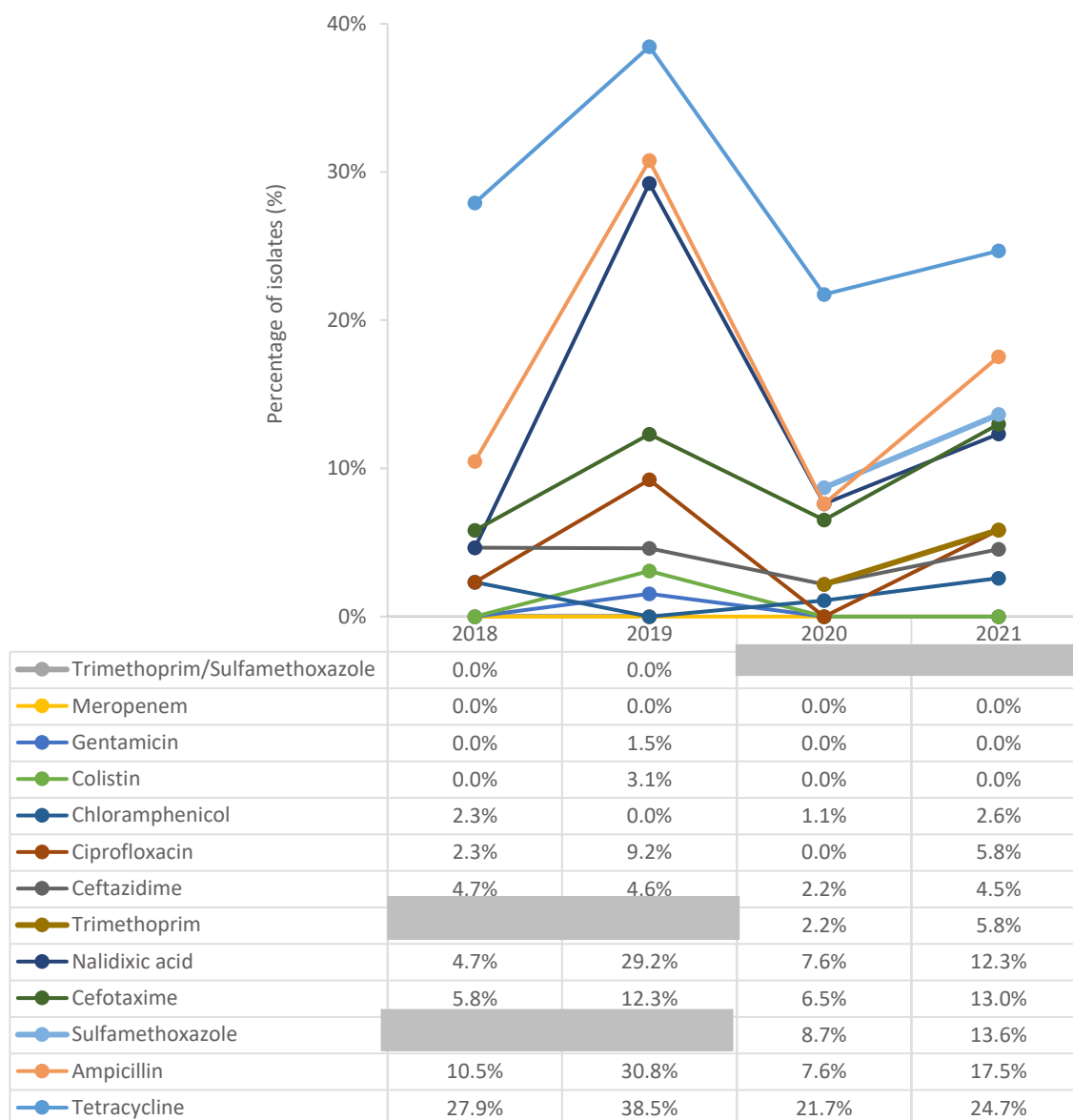


Note: Starting in 2020, the monitoring of resistance uses the Sensititre™ Asia Surveillance Plates (ASSECAF and ASSECB; see Appendix 2), which monitors trimethoprim and sulfamethoxazole resistance separately.

Ruminant farms

Overall, there was an uptick in percentage resistance in 2021 after a decrease in 2020 (Figure 42). In 2020, *E. coli* isolates from ruminant farms (n=92) were most frequently resistant to tetracycline, followed by sulfamethoxazole, ampicillin and nalidixic acid, while fully susceptible to ciprofloxacin, colistin, gentamicin and meropenem. In 2021, isolates (n=154) were most frequently resistant to tetracycline, followed by ampicillin, sulfamethoxazole, cefotaxime and nalidixic acid; all were susceptible to colistin, gentamicin and meropenem.

Figure 42. Percentage resistance of *E. coli* isolated in local ruminant farms, 2018-2021



Note: Starting in 2020, the monitoring of resistance uses the Sensititre™ Asia Surveillance Plates (ASSECAF and ASSECB; see Appendix 2), which monitors trimethoprim and sulfamethoxazole resistance separately.

Similar to the trends observed for *Salmonella* in local poultry farms, there were notable increases in the percentage of *E. coli* isolates resistant to sulfamethoxazole and trimethoprim in local poultry and ruminant farms in 2020 and 2021. Furthermore, several *E. coli* isolates were resistant to cefotaxime and ceftazidime in local poultry and ruminant farms. Cefotaxime and ceftazidime are considered "extended spectrum" cephalosporins, and resistance to these antibiotics is often used as an indicator of ESBL production¹⁷. ESBL-producing bacteria are a concern in healthcare settings because they can limit the treatment options available for infections, making them more challenging to manage. Investigations to establish whether these isolates are ESBL-producing are currently in progress and will be documented in the next report.

In Imported and Retail Food Products

Extended Spectrum Beta-lactamase-producing E. coli (ESBL-Ec) in Imported Food Products

ESBL-Ec can be found in food-producing animals and may also be present in food products as a result of extraneous contamination along the food supply chain, such as during handling or processing.

Under SFA's AMR surveillance programme, a total of 366 ESBL-Ec isolates was obtained from 2575 imported, local slaughterhouses, abattoir and aquatic farm food products tested in 2020 and 2021. ESBL-Ec was most frequently isolated from import and retail poultry products (Table 7), with detection rates over 50% in 2020 and over 30% in 2021. In comparison, prevalence of ESBL-Ec in pork and pork products was lower, ranging from 4.1% to 8.8%.

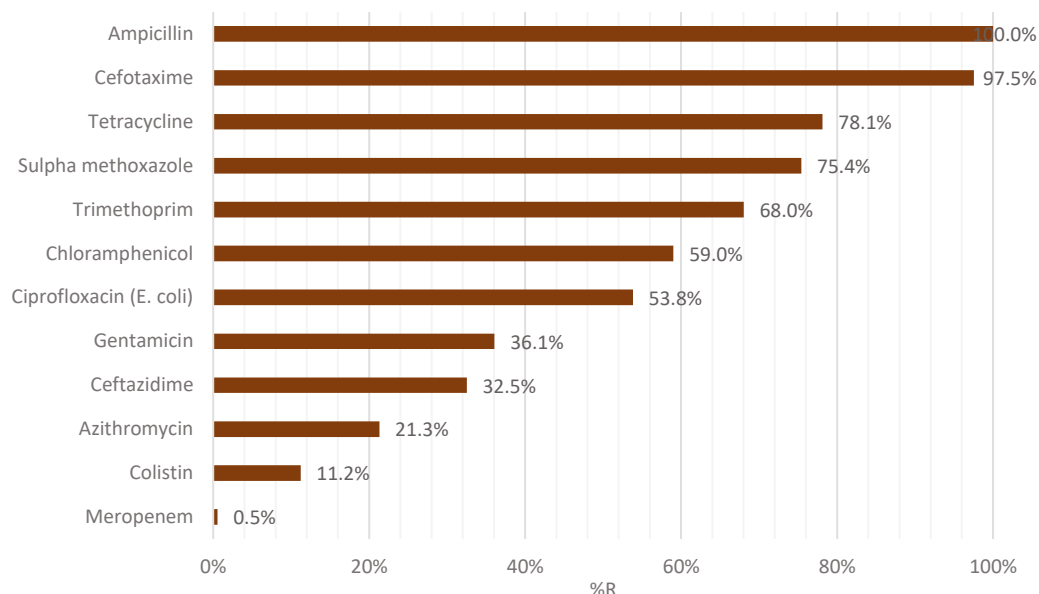
Over 75% of the ESBL-Ec isolates tested were concurrently resistant to ampicillin, cefotaxime, tetracycline and sulfamethoxazole (Figure 43).

Table 7. Percentage of *ESBL Ec* detection in poultry and pork products, 2020 – 2021

<i>Product type</i>	2020	2021
Imported and food establishments' poultry products	53.3% (105/197)	38.0% (184/485)
Retail poultry products	59.4% (98/165)	39.4% (180/457)
Imported pork and abattoir products	4.1% (5/122)	5.7% (10/176)
Retail pork products	4.3% (6/140)	8.8% (33/375)

¹⁷ Rawat D, Nair D. Extended-spectrum β -lactamases in Gram Negative Bacteria. J Glob Infect Dis. 2010 Sep;2(3):263-74. doi: 10.4103/0974-777X.68531. PMID: 20927289; PMCID: PMC2946684.

Figure 43. Percentage resistance (%R) of ESBL *Ec* isolates (n=366) from imported, local slaughterhouses, abattoir and fish farm samples, 2020-2021



Extended Spectrum Beta-lactamase-producing E. coli (ESBL-Ec) in Retail Food Products

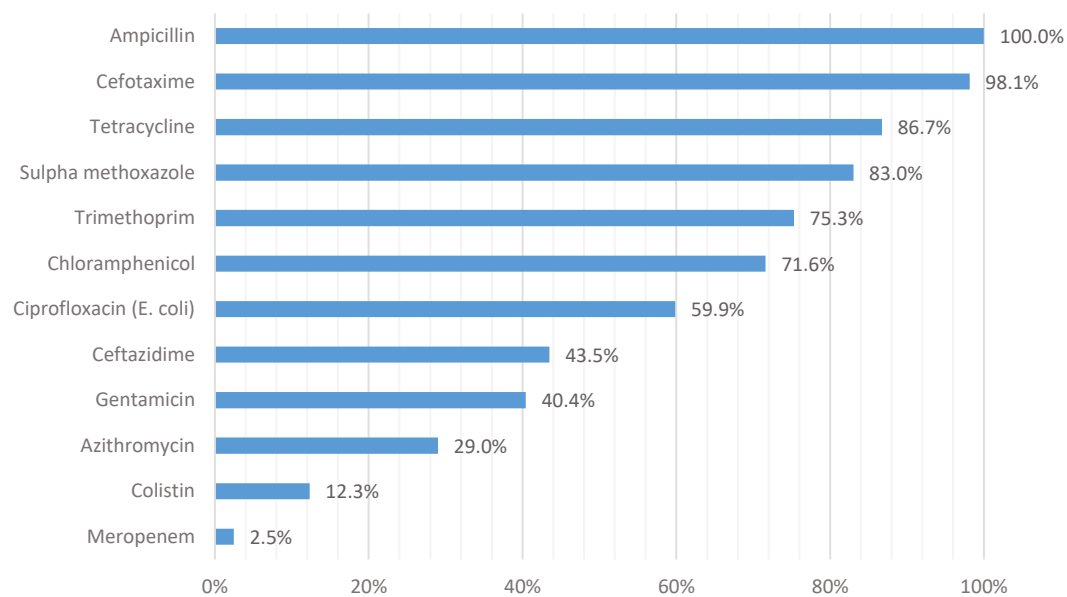
Under the SFA's AMR surveillance programme in 2020 and 2021, a total of 324 *ESBL Ec* isolates were obtained from 1728 retail raw food, such as beef, fish, lamb, poultry, pork and seafood products. Among the isolates tested, over 70% were resistant to ampicillin, cefotaxime, tetracycline and sulfamethoxazole, trimethoprim and chloramphenicol (Figure 44).

Retail meat products had higher rates of detection than products sampled upstream at points of import, processing (food establishments) and farms (Table 8), suggesting possibility of extraneous contamination along the supply chain leading to retail. This, and similar findings for *Salmonella* spp, could signal a need to strengthen the cold chain management in the food chain.

Table 8. Percentage of ESBL-Ec detected in retail food products vs imported products and food establishments, 2020 – 2021.

Source type	2020	2021
Imported, food establishments, farm products	15.7% (117/745)	13.6% (249/1830)
Retail meat products	22.3% (98/439)	17.5% (226/1289)

Figure 44. Percentage resistance (%R) of ESBL-Ec isolates (n=324) from retail raw meat samples, 2020-2021



Antimicrobial Resistance in Bacteria from Companion Animals and Wildlife

AMR surveillance in companion animals

AMR is a pressing global health issue with implications for both human and animal populations. However, despite the frequent use of antimicrobials in companion animals and their regular contact with humans, the role of companion animals in AMR remains inadequately understood¹⁸. National AMR surveillance programmes in companion animals are therefore essential, focusing on bacteria of concern to public and animal health. NParks' surveillance of companion animals comprises both passive and active surveillance, and includes *E. coli*, *Klebsiella pneumoniae*, methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). Bacteria isolated are subjected to AST against clinically and epidemiologically important antimicrobial agents according to CLSI standards (Appendix II).

E. coli, ubiquitous in the intestinal tract of humans and animals. The organism can acquire various AMR genes, leading to the emergence of MDR strains. Resistant *E. coli* in companion animals poses a potential risk of transmission to humans, especially through close contact.

K. pneumoniae, an opportunistic pathogen causing infections in various hosts, including companion animals, presents challenges due to its ability to develop resistance to multiple antibiotics, including beta-lactams.

MRSA and **MRSP** are frequently associated with skin, wound, or surgical site infections, otitis, and urinary tract infections in cats and dogs. While MRSA is often acquired from humans, serving as potential bacterial reservoirs, MRSP is more commonly isolated in dogs and cats. MRSP tends to colonise dogs, posing a significant risk of zoonotic transmission. Although human infections of MRSP are rare¹⁹, both MRSA and MRSP are concerning due to limited treatment options for infections by these organisms and the potential for transmission between animals and humans.

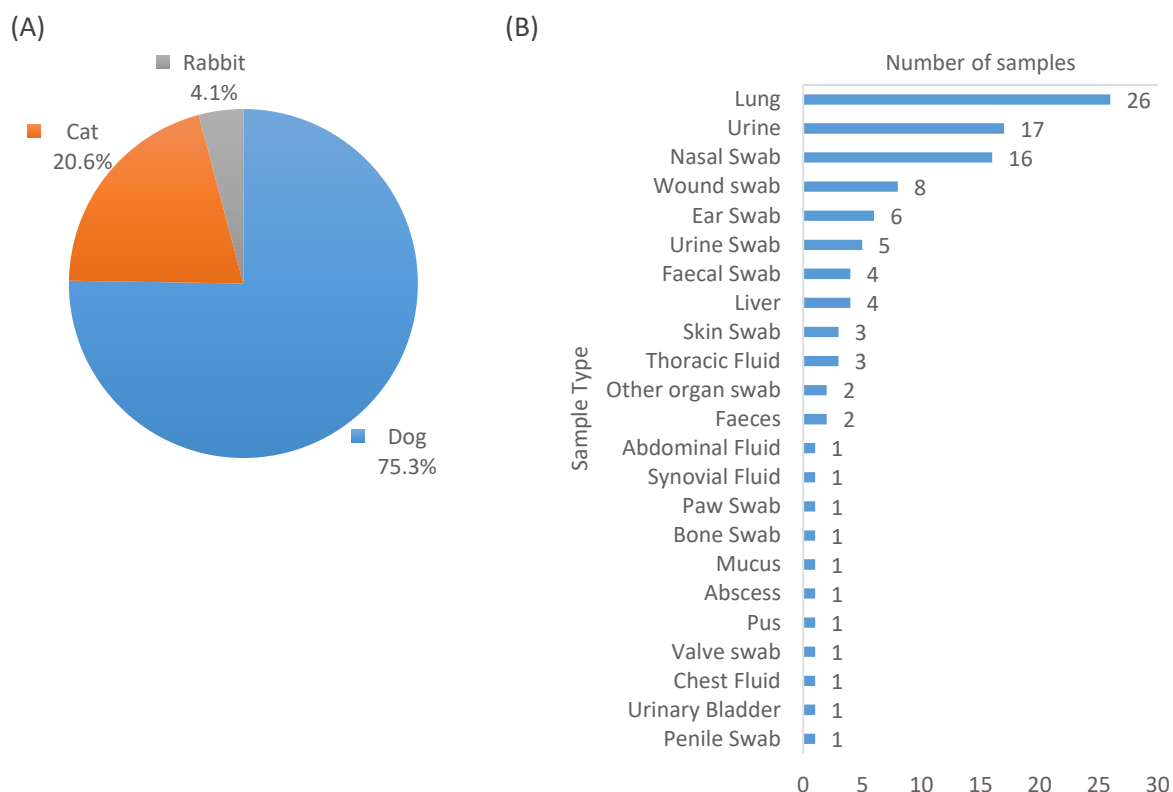
Sick companion animals

NParks has been conducting passive AMR surveillance on sick companion animals. In 2020, the surveillance focused on *E. coli*, *K. pneumoniae*, MRSA and MRSP from clinical samples submitted by veterinarians. In 2020 and 2021, the samples were mainly collected from dogs (75.3%), followed by cats (20.6%) and rabbits (4.1%) (Figure 45A). Lung (24.3%), urine (15.9%) and nasal swab (15.0%) were the most common sample types received by the laboratory (Figure 45B).

¹⁸ Damborg P., Broens E.M., Chomel B.B., Guenther S., Pasmans F., Wagenaar J.A., Weese J.S., Wieler L.H., Windahl U., Vanrompay D., et al. Bacterial Zoonoses Transmitted by Household Pets: State-of-the-Art and Future Perspectives for Targeted Research and Policy Actions. J. Comp. Pathol. 2016;155:S27–S40.

¹⁹ Reflection paper on methicillin-resistant *Staphylococcus pseudintermedius*. EMA/CVMP/SAGAM/736964/ 2009 Committee for Medicinal Products for Veterinary Use (CVMP), 20 September 2010.

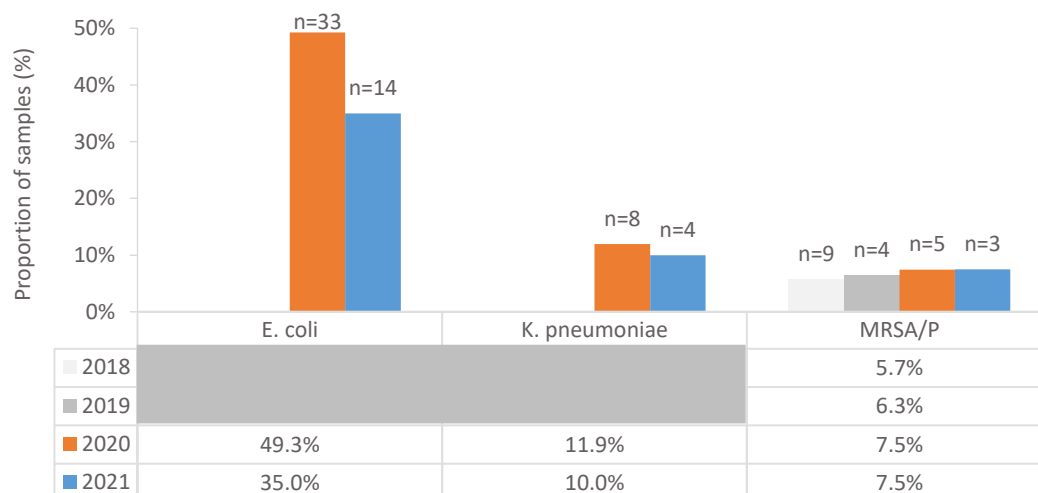
Figure 45. Distribution of samples for passive AMR surveillance on sick companion animals by (A) species and (B) sample type, 2020-2021



E. coli, *K. pneumoniae* and MRSP in companion animals

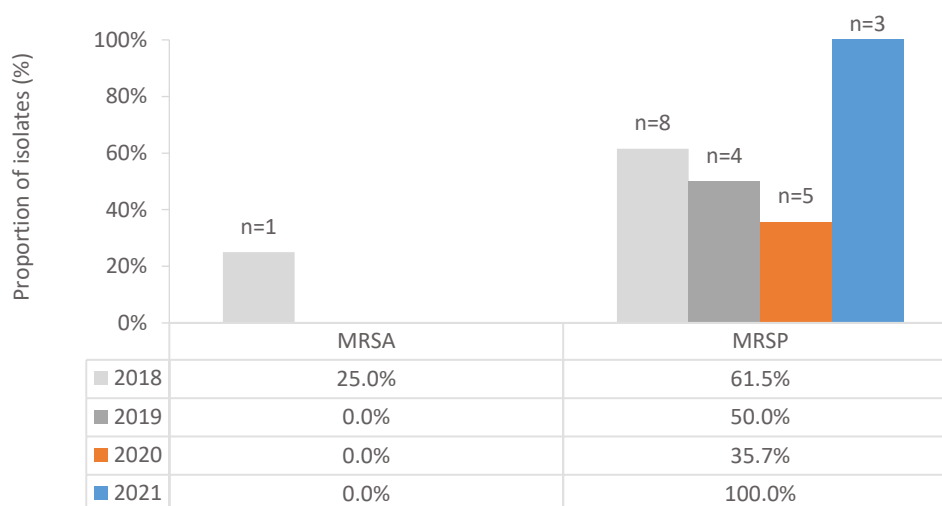
In 2020, *E. coli* and *K. pneumoniae* were isolated from 33 (49.3%) and 8 (11.9%) companion animals, respectively. In 2021, *E. coli* and *K. pneumoniae* were isolated from 14 (35.0%) and 4 (10.0%) companion animals, respectively (Figure 46A).

Figure 46A. Proportion of *E. coli*, *K. pneumoniae* and MRSA/P isolated from sick companion animals, 2018-2021 (n = number of samples)



In 2020 and 2021, three *S. aureus* were isolated in companion animals and all were susceptible to methicillin (cefoxitin; Figure 46B). In 2020, 5/14 (35.7%) *S. pseudintermedius* isolated in companion animals were MRSP. In 2021, all three *S. pseudintermedius* isolated from sick companion animals were found to be MRSP, representing 7.5% of sampled companion animals (Figure 46A).

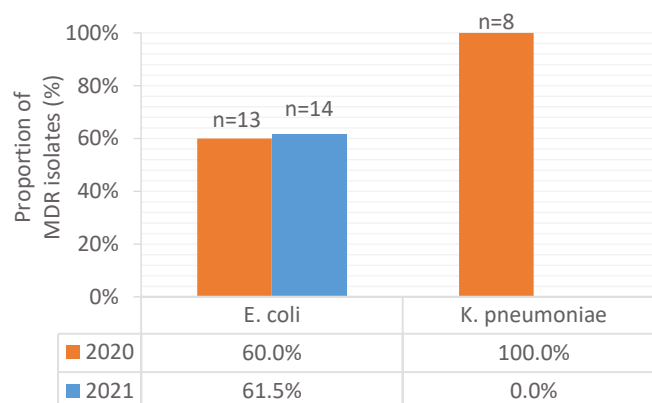
Figure 46B. Proportion of *S. aureus* and *S. pseudintermedius* that were methicillin resistant in sick companion animals, 2018-2021 (n= number of isolates)



MDR rates of companion animal isolates

The proportion of MDR *E. coli* isolated from companion animals were 60.0% and 61.5% in 2020 and 2021, respectively. All eight *K. pneumoniae* isolated in 2020 were found to be MDR, while none of the four isolated in 2021 were MDR. However, the small numbers of isolates (<30) fall below the threshold of reliability for estimating resistance proportions. All eight MRSP isolated in 2020 and 2021 were MDR.

Figure 47. Proportion of MDR *E. coli* and *K. pneumoniae* in sick companion animals, 2020-2021 (n = number of isolates)

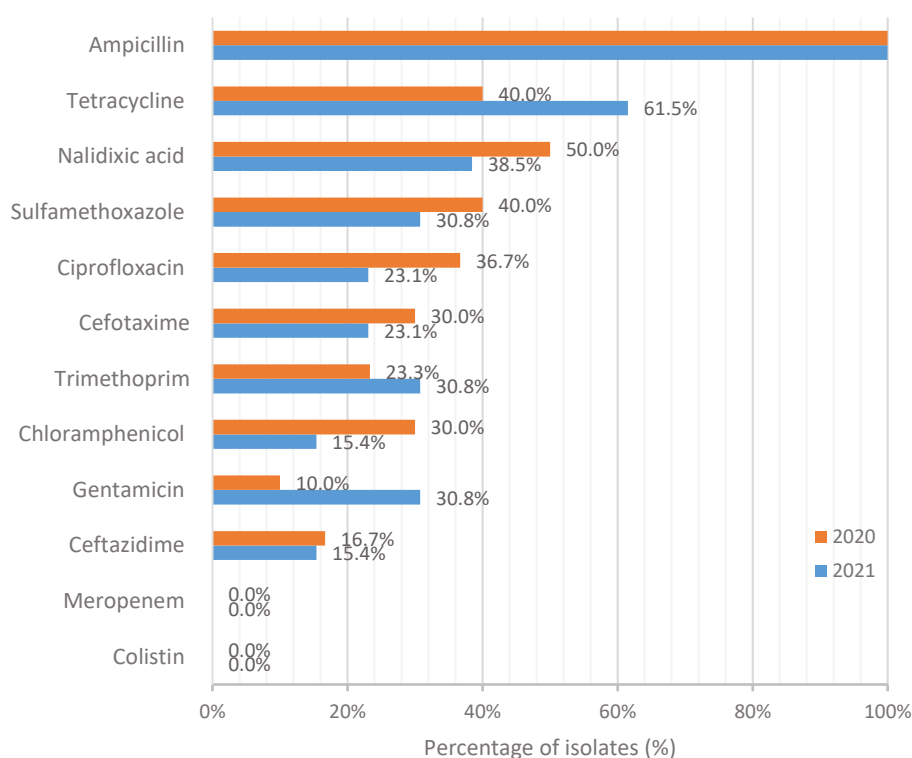


E. coli from sick companion animals

All *E. coli* isolated from sick companion animals in 2020 (n=33) and 2021 (n=14) were resistant to ampicillin but susceptible to colistin and meropenem. In 2020, some *E. coli* isolates were resistant to nalidixic acid (50.0%), tetracycline (40.0%) and sulfamethoxazole (40.0%).

In 2021, some *E. coli* isolates were resistant to tetracycline (61.5%) and nalidixic acid (38.5%). Resistance to ciprofloxacin decreased from 36.7% in 2020 to 23.1% in 2021, while resistance to ceftazidime, a third-generation cephalosporin, remained stable at 15.4% in 2021. Percentage resistance to tetracycline, gentamicin and trimethoprim increased in 2021 compared to 2020, and the percentage resistance to nalidixic acid, sulfamethoxazole, ciprofloxacin, cefotaxime and chloramphenicol decreased (Figure 48).

Figure 48. Percentage resistance of *E. coli* isolated from sick companion animals, 2020-2021

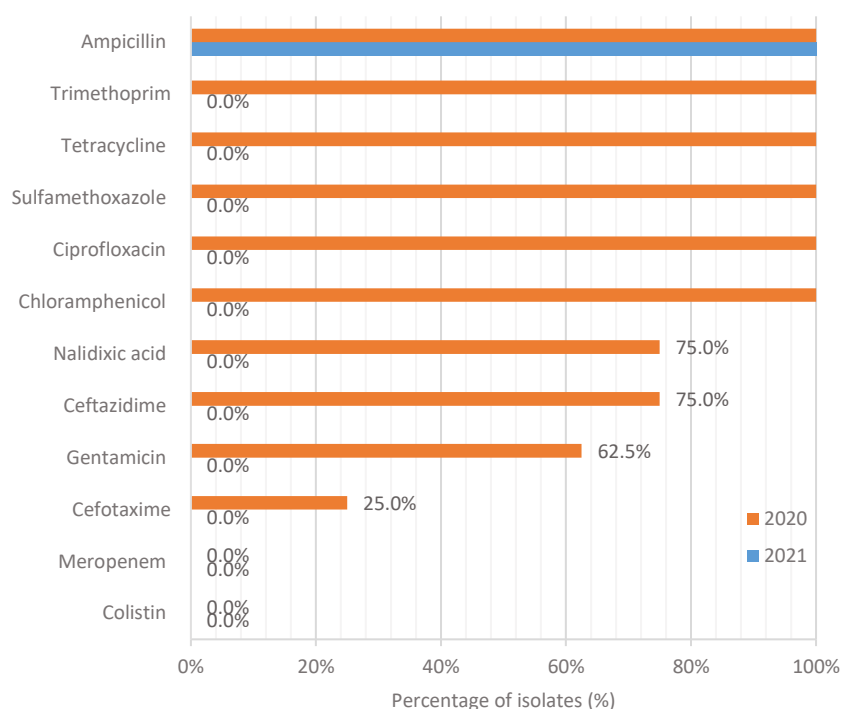


Note: Bars without data labels represent 100% of isolates being resistant.

K. pneumoniae from sick companion animals

All *K. pneumoniae* (n=8) isolated from sick companion animals in 2020 were resistant to ampicillin, trimethoprim, tetracycline, sulfamethoxazole, ciprofloxacin, and chloramphenicol but susceptible to colistin and meropenem. In 2020, 75% of the *K. pneumoniae* isolates were resistant to nalidixic acid and ceftazidime, 62.5% were resistant to gentamicin, and 25.0% were resistant to cefotaxime.

In contrast, *K. pneumoniae* isolated in 2021 (n=4) were resistant only to ampicillin and susceptible to all other antimicrobials tested (Figure 49).

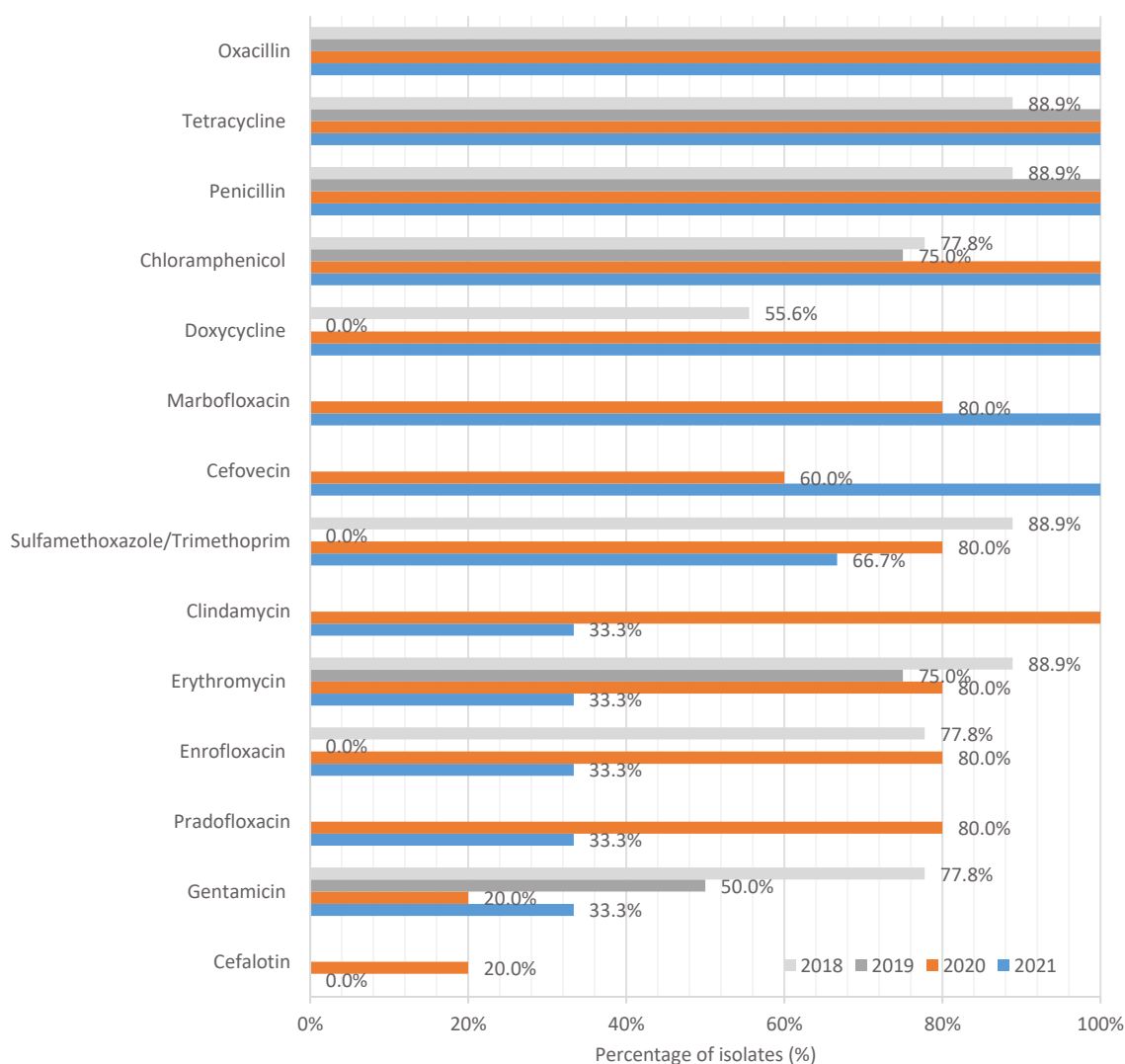
Figure 49. Percentage resistance of *K. pneumoniae* isolated from sick companion animals, 2020-2021

Note: Bars without data labels represent 100% of isolates being resistant.

MRSP from sick companion animals

Concurrent resistance to other antibiotics is frequently seen with MRSP and MRSA. In 2020, all clinical MRSP isolates (n=5) exhibited resistance to oxacillin, tetracycline, penicillin, chloramphenicol, doxycycline, and clindamycin; 80% were resistant to erythromycin, trimethoprim/sulfamethoxazole, enrofloxacin, marbofloxacin, and pradofloxacin, while 60% were resistant to cefovecin, and 20% were resistant to gentamicin and cefalotin.

In 2021, all MRSP isolates (n=3) were resistant to oxacillin, tetracycline, penicillin, chloramphenicol, doxycycline, marbofloxacin, and cefovecin; 66.7% of them were resistant to trimethoprim/sulfamethoxazole; while 33.3% were resistant to erythromycin, gentamicin, enrofloxacin, clindamycin and pradofloxacin (Figure 50). All isolates were susceptible to nitrofurantoin in both 2020 and 2021 (data not shown).

Figure 50. Percentage resistance of MRSA and MRSP from sick companion animals, 2018-2021

Note: AST was performed using disk diffusion prior to 2020. Starting in 2020, the monitoring of resistance has involved the utilisation of VITEK AST GP80 cards (see Appendix 2), expanding the antimicrobial panel to include veterinary important antimicrobials relevant to companion animals like nitrofurantoin, cefalotin, cefovecin, pradofloxacin, marbofloxacin and clindamycin. Bars without data labels represent 100% of isolates being resistant.

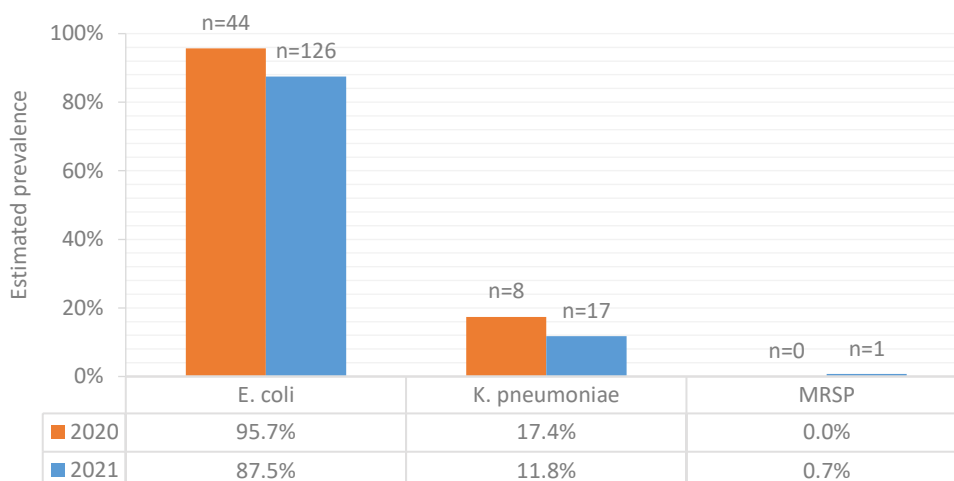
AMR surveillance on free-roaming dogs

In 2020, NParks embarked on active AMR surveillance programme targeting *E. coli*, *K. pneumoniae*, MRSA and MRSP in healthy free-roaming dogs across Singapore. Singapore has an estimated population of 2,200 free-roaming dogs that inhabit locations close to human communities. Since 2018, free-roaming dogs have been sterilised and either rehomed or released into the environment under NParks' nationwide Trap-Neuter-Release/Rehoming-Manage (TNRM) Programme.

The free-roaming dogs were sampled from those admitted into the TNRM programme. In 2020, *E. coli* and *K. pneumoniae* were isolated from 44 (95.7%) and 8 (17.4%) free-roaming dogs, respectively. All 18 *S. pseudintermedius* isolated in free-roaming dogs in 2020 were found susceptible to methicillin

(oxacillin). In 2021, *E. coli* and *K. pneumoniae* were isolated from 126 (87.5%) and 17 (11.8%) free-roaming dogs, respectively (Figure 51). Out of the 55 *S. pseudintermedius* isolated in free-roaming dogs in 2021, 1 (1.8%) was MRSP, accounting for 0.7% of the free-roaming dog population sampled in 2021. *Staphylococcus aureus* and MRSA was not isolated in free-roaming dogs in 2020 and 2021.

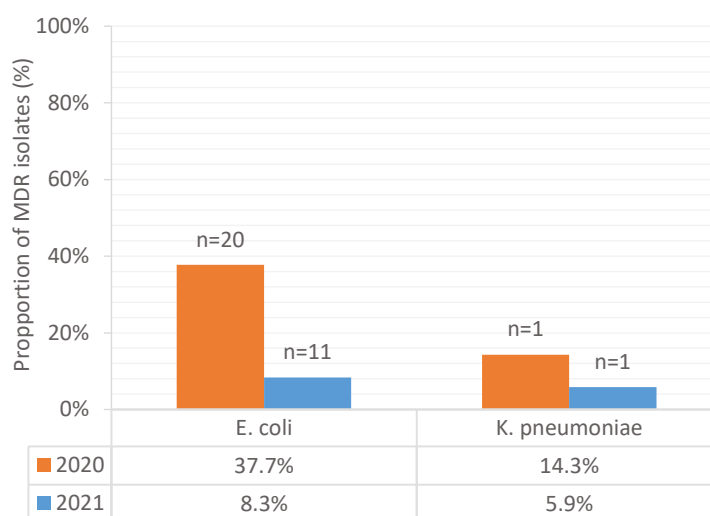
Figure 51. Estimated prevalence of *E. coli*, *K. pneumoniae* and MRSP in local free-roaming dog populations, 2020-2021 (n = number of samples with bacteria isolated)



Resistance profiles of E. coli, K. pneumoniae and MRSP isolated from free-roaming dogs

Although this population of dogs was less likely to have been fed or treated with antibiotics, MDR bacteria were still recovered from their samples (Figure 52). The proportion of MDR *E. coli* isolated from local free-roaming dogs decreased from 37.7% in 2020 to 8.3% in 2021, while that of MDR *K. pneumoniae* decreased from 14.3% in 2020 to 5.9% in 2021. The MRSP isolated in 2021 was MDR.

Figure 52. Proportion of MDR *E. coli* and *K. pneumoniae* in local free-roaming dog populations, 2020-2021 (n = number of isolates)

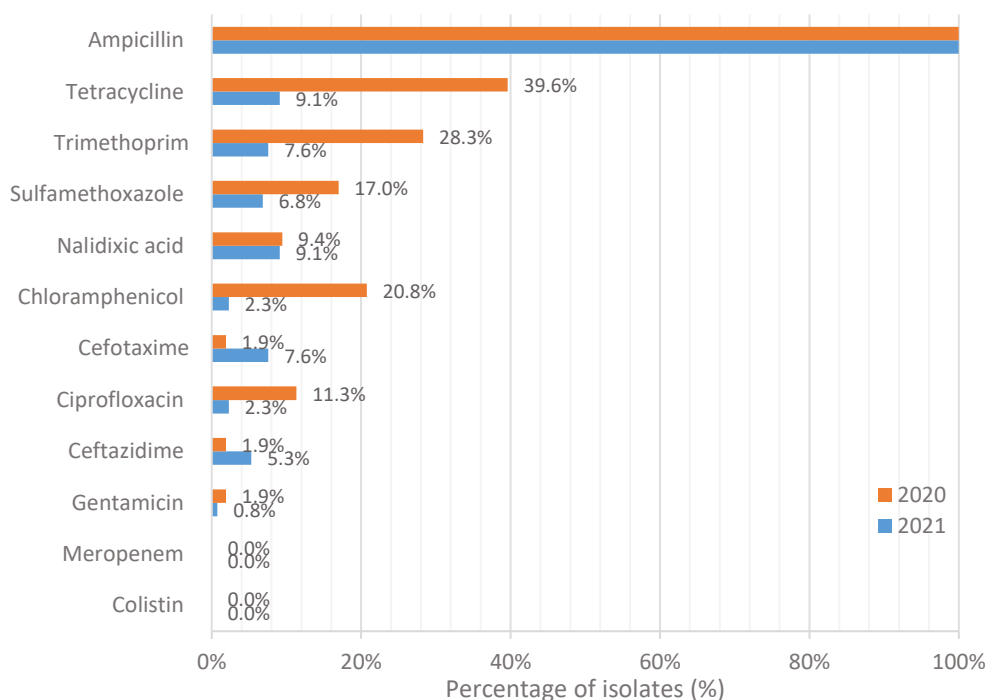


E. coli in free-roaming dogs

Similar to the *E. coli* isolated in sick companion animals, *E. coli* isolated in 2020 (n=44) and 2021 (n=126) from free-roaming dogs exhibited resistance to ampicillin (100%) but were fully susceptible to colistin and meropenem. The high proportion of isolates resistant to ampicillin in *E. coli* from dogs was consistent with rates reported elsewhere, such as in Thailand²⁰ and Japan²¹. The percentage resistance of *E. coli* isolated from free-roaming dogs were generally lower than those isolated from sick companion animals (Figure 48).

The resistance percentages of *E. coli* isolates from 2021 were generally lower than those isolated in 2020. In 2020, some *E. coli* isolates from free-roaming dogs were resistant to tetracycline (39.6%), trimethoprim (28.3%), and chloramphenicol (20.8%) (Figure 53). In 2021, there were reductions in the percentage of resistance to several antibiotics including tetracycline, trimethoprim, chloramphenicol, sulfamethoxazole. However, resistance percentage for cefotaxime increased.

Figure 53. Percentage resistance of *E. coli* isolated from free-roaming dogs, 2020-2021



Note: Bars without data labels represent 100% of isolates being resistant.

K. pneumoniae in free-roaming dogs

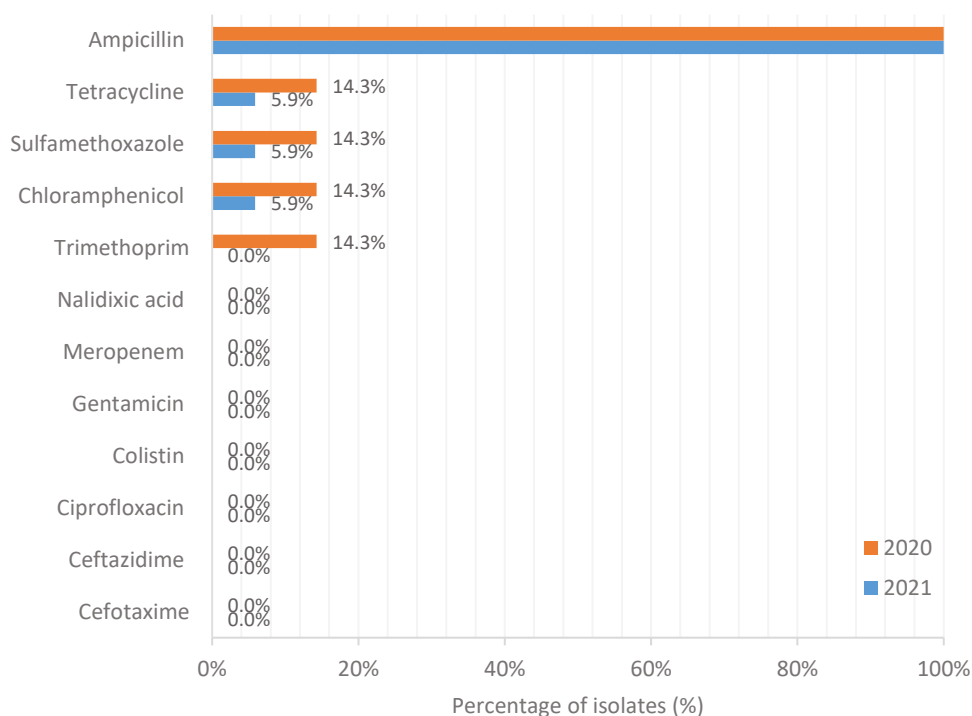
All *K. pneumoniae* isolated from free-roaming dogs in 2020 (n=44) and 2021 (n=126) exhibited resistance to ampicillin but were fully susceptible to cefotaxime, ceftazidime, ciprofloxacin, colistin, gentamicin, meropenem and nalidixic acid. In 2020, the percentage of *K. pneumoniae* isolates resistant

²⁰ Buranasinsup et al, 2023. Prevalence and characterisation of antimicrobial-resistant *Escherichia coli* isolated from veterinary staff, pets and pet owners in Thailand. *Journal of Infections and Public Health*, 16(1):194-202

²¹ Hata et al, 2022. Surveillance of antimicrobial *Escherichia coli* in Sheltered dogs in the Kanto Region of Japan. *Sci Rep*, 12:773.

to tetracycline, sulfamethoxazole, chloramphenicol and trimethoprim were 14.3%. In 2021, the percentage of *K. pneumoniae* isolates resistant to tetracycline, sulfamethoxazole and chloramphenicol were 5.9%, while all *K. pneumoniae* isolates were resistant to trimethoprim.

Figure 54. Percentage resistance of *K. pneumoniae* isolated from free-roaming dogs, 2020-2021



Note: Bars without data labels represent 100% of isolates being resistant.

MRSP in free-roaming dogs

MRSP are typically resistant to multiple antibiotics. The sole MRSP isolate isolated from a free-roaming dog in 2021 was resistant to penicillin, oxacillin, ceftiofur, gentamicin, enrofloxacin, marbofloxacin, pradofloxacin, erythromycin, clindamycin, doxycycline, tetracycline and chloramphenicol and susceptible to cefalotin, nitrofurantoin and trimethoprim (data not shown).

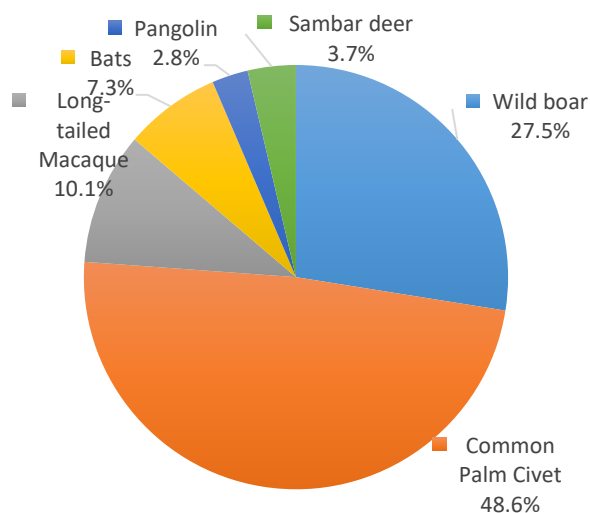
AMR surveillance in wildlife

AMR in wildlife is a growing concern due to its potential impact on ecosystem health and public health²². Wildlife can serve as reservoirs of AMR, harbouring resistant bacteria that may be transferred to other animals or humans. Despite an increasing awareness of this issue, there remains a lack of knowledge in this field, hindering our understanding of the dynamics of AMR among wildlife populations.^{23,24}

NPark's wildlife AMR surveillance is passive and targets *E. coli* isolated from wild animals. *E. coli*, being a common bacterium found in the intestines of many animals, is often the target bacterium for studying AMR in wildlife.²⁵ Monitoring and studying AMR in wildlife can aid our understanding in AMR transmission pathways in Singapore's small island state context, where urban and non-urban areas are in close proximity, and where necessary to develop strategies to mitigate the spread of AMR from wildlife to other environments, and vice versa.

In 2020 and 2021, samples were collected opportunistically from a total of 109 animals. The highest number of samples were obtained from the common palm civet (48.6%), followed by wild boar (27.5%), long-tailed macaque (10.1%), bats (7.3%), sambar deer (3.7%) and pangolin (2.8%) (Figure 55). Bacteria isolated were subjected to AST against clinically and epidemiologically important antimicrobial agents.

Figure 55. Distribution of samples for passive AMR surveillance on wildlife, 2020-2021



²² Arnold, K. E., Williams, N. J., & Bennett, M. (2016). 'Disperse abroad in the land': the role of wildlife in the dissemination of antimicrobial resistance. *Biology Letters*, 12(8), 20160137.

²³ Huijbers PMC, Blaak H, de Jong MCM, Graat EAM, Vandenbroucke-Grauls CMJE, Husman AMDR. 2015 Role of the environment in the transmission of antimicrobial resistance to humans: a review. *Environ. Sci. Technol.* **49**, 11 993–12 004. (doi:10.1021/acs.est.5b02566)

²⁴ Greig J, Rajic A, Young I, Mascarenhas M, Waddell L, LeJeune J. 2015 A scoping review of the role of wildlife in the transmission of bacterial pathogens and antimicrobial resistance to the food chain. *Zoonoses Public Health* **62**, 269–284. (doi:10.1111/zph.12147)

²⁵ Lagerstrom, K. M., & Hadly, E. A. (2021). The under-investigated wild side of *Escherichia coli*: genetic diversity, pathogenicity and antimicrobial resistance in wild animals. *Proceedings of the Royal Society B*, 288(1948), 20210399.

Resistance profile of E. coli from wildlife

E. coli was isolated from 59/62 (95.2%) and 39/47 (83.0%) wild animals in 2020 and 2021, respectively (Figure 56A). Of these, one *E. coli* isolated in 2020 and two *E. coli* isolated in 2021 were found to be MDR (Figure 56B).

AST of *E. coli* isolated in wildlife in 2020 and 2021 revealed distinct AMR patterns; they were less frequently resistant to antimicrobials tested compared to *E. coli* from food-producing and companion animals. All isolates were susceptible to ceftazidime, ciprofloxacin, gentamicin and meropenem. Isolates from 2020 were susceptible to cefotaxime; 8.5% were resistant to tetracycline, 5.1% were resistant to sulfamethoxazole, 3.4% were resistant to ampicillin and 1.7% were resistant to chloramphenicol, colistin and nalidixic acid.

Figure 56. Proportion of (A) *E. coli* and (B) MDR *E. coli* isolated from wildlife, 2020-2021

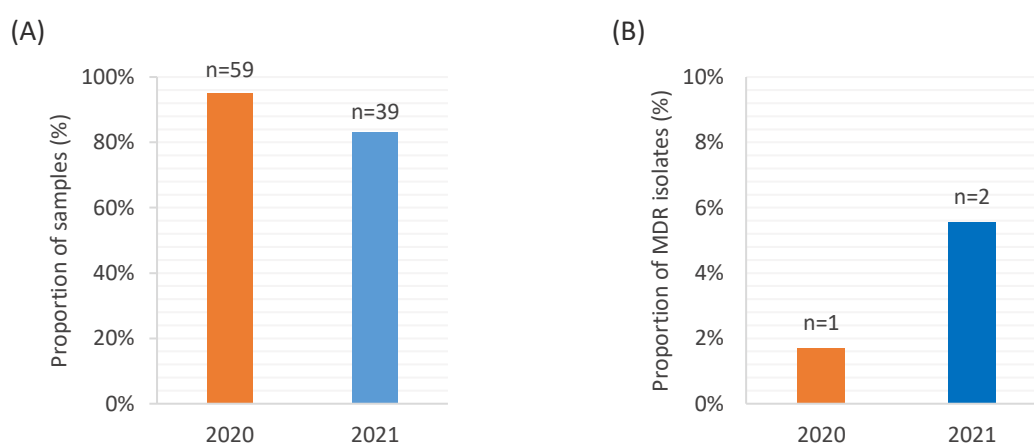
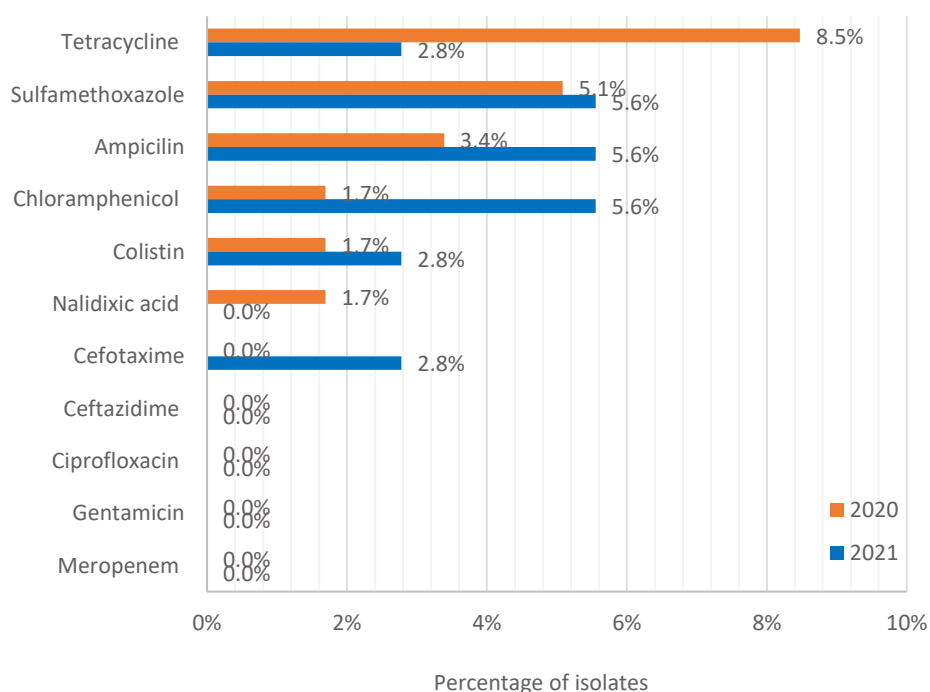


Figure 57. Percentage resistance of *E. coli* isolated from wildlife, 2020-2021



Discussion

Collectively, the AMR profiles of *E. coli* described here provide an inaugural baseline of AMR in wildlife in Singapore. The distinct pattern and lower percentage of resistance in wildlife may suggest that AMR in wildlife is less prevalent compared to other animal sectors with greater exposure to anthropogenic activity, such as food-producing and companion animals.

The data here does not represent the entire distribution of the AMR bacteria. Further study would be needed to assess the impact of AMR in wildlife on public and animal health over a longer time frame. This underscores the importance of refining the ongoing monitoring, such as having regular AMR testing at specified timepoints within a time frame and/or with distributions mapped out, to better understand and address AMR dynamics in wildlife.

Antimicrobial Resistant Bacteria in the Environment

The environment may act as a transmission pathway and reservoir for antimicrobial resistance genes (ARG) and AMR bacteria (ARB). In Singapore, several studies have been conducted to better understand the prevalence of AMR in the environment. The presence of chemicals or antibiotic resistance genes in selected waterways, reservoirs and coastal environments were also previously investigated.^{26 27} Previous studies conducted by NEA revealed the presence of antimicrobial resistant *Enterococcus* spp. and *Pseudomonas aeruginosa* from recreational beach environments and man-made water features, respectively²⁸.

Arising from the detection of AMR bacteria in these studies, it was needful to understand the baseline levels of AMR bacteria and possible contamination sources in various water environments. To this end, NEA conducted spatiotemporal surveillance studies in different water environments and four bacterial species of interest *Enterococcus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa* and *E. coli* were selected as key indicators to track AMR.

Study 1: Screening of Extended-Spectrum Beta Lactamase *E. coli* in recreational beach waters in Singapore

Extended spectrum beta-lactamase *Escherichia coli* (ESBL-Ec) has been identified by the WHO as an indicator for integrated multi-sectoral AMR surveillance for monitoring its prevalence and trend globally. Previous studies in the Netherlands in 2011-2012²⁹, Croatia in 2009-2013³⁰ and Norway in 2010³¹ had found a low prevalence of ESBL-Ec in recreational beach waters, with rates of 1%, 7.7% and 3.8%, respectively. In non-clinical settings in Singapore, snapshot studies had detected ESBL-Ec in wild birds³², reservoirs²⁸ and aquaculture²⁷ sites. To understand further the AMR burden in recreational beach water, a study was conducted involving a total of 90 water samples from six different recreational beaches across Singapore, collected over three different time periods.

²⁶ Ng, C., H. Chen, S. G. Goh, L. Haller, Z. Wu, F. R. Charles, A. Trottet and K. Gin (2018). "Microbial water quality and the detection of multidrug resistant *E. coli* and antibiotic resistance genes in aquaculture sites of Singapore." *Mar Pollut Bull* 135: 475.

²⁷ Zhong, Y., S. Guo, K. L. G. Seow, G. O. H. Ming and J. Schlundt (2021). "Characterization of Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* Isolates from Jurong Lake, Singapore with Whole-Genome-Sequencing." *Int J Environ Res Public Health* 18: 937.

²⁸ One Health Report on Antimicrobial Use and Resistance, 2019.

²⁹ Blaak, H., P. d. Kruijf, R. A. Hamidjaja, A. H. A. M. v. Hoek, A. M. d. R. Husman and F. M. Schets (2014). "Prevalence and characteristics of ESBL-producing *E. coli* in Dutch recreational waters influenced by wastewater treatment plants." *Vet Microbiol* 171: 448.

³⁰ Maravić, A., M. Skočibušić, S. Cvjetan, I. Šamanić, Ž. Fredotović and J. Puizina (2015). "Prevalence and diversity of extended-spectrum-β-lactamase-producing Enterobacteriaceae from marine beach waters." *Mar Pollut Bull* 90: 60.

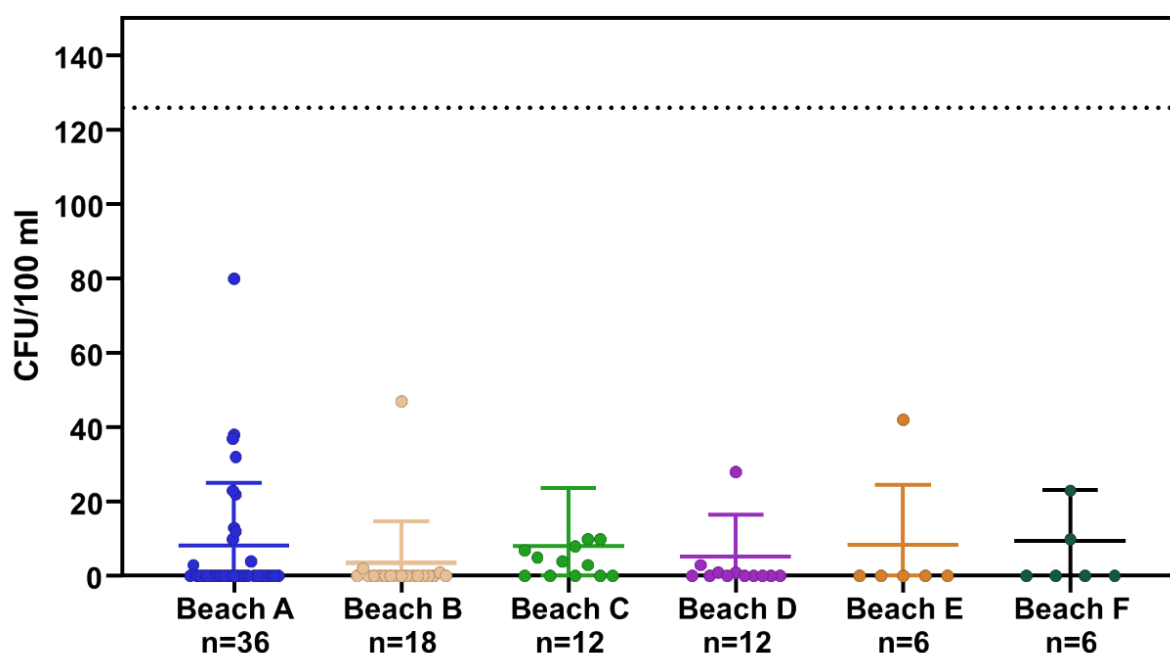
³¹ Jørgensen, S. B., A. V. Søråas, L. S. Arnesen, T. M. Leegaard, A. Sundsfjord and P. A. Jenum (2017). "A comparison of extended spectrum β-lactamase producing *Escherichia coli* from clinical, recreational water and wastewater samples associated in time and location." *PLoS One* 12: e0186576

³² Ong K.H., Khor W.C., Quek J.Y., et al (2020). "Occurrence and Antimicrobial Resistance Traits of *Escherichia coli* from Wild Birds and Rodents in Singapore". *Int. J. Environ. Res. Public Health* 17(15):5606.

Enumeration of bacteria present in beach water

Enumeration of total *E. coli* in the water samples using the APHA 9223 B & H standard method showed that only 28/90 (31.3%) samples had *E. coli*. Total *E. coli* counts ranged from 1 to 80 CFU/100 ml (Figure 58) which were within limits of US EPA and EU guidelines for recreational waters. One site from Beach C had continuous positive *E. coli* detection in samples collected over three different sampling dates. A few sites at Beach A (n = 3), Beach B (n = 3), Beach D (n = 1) and Beach E (n = 1) yielded no *E. coli*. The variable distribution suggests that *E. coli* population at the recreational beaches in Singapore may be transient.

Figure 58. Total *E. coli* (CFU/100 ml) for all collected water samples from recreational beaches.



Note: All counts were within the limits of ≤ 126 CFU/100 mL (signified by the dotted line) and ≤ 500 CFU/100 mL, as stipulated in the US EPA and EU guidelines respectively for recreational beach waters.

ESBL-EC was not detected in water samples

No ESBL-Ec was isolated from the collected water samples based on direct plating on CHROMID ESBL agar; and pre-enrichment with ceftriaxone prior to plating on CHROMID ESBL agar. Those that grew on CHROMID ESBL agar were *Pseudomonas* spp. (n = 5), *Enterobacter* spp. (n = 5), *Proteus* spp. (n = 5) and *Deftia* spp. (n = 4). None of the *Pseudomonas* spp. and *Enterobacter* spp. were true ESBL when tested using double disc synergy test. Ceftriaxone-resistant isolates (n=20) were obtained from enrichment broth supplemented with ceftriaxone. However, none of them were *E. coli*. Only culture-based approach was utilised to determine the presence of ESBL-Ec. Inclusion of molecular methods such as quantitative PCR and metagenomics would complement and further support the findings.

Regular monitoring at different seasons required to understand the prevalence of ESBL-Ec

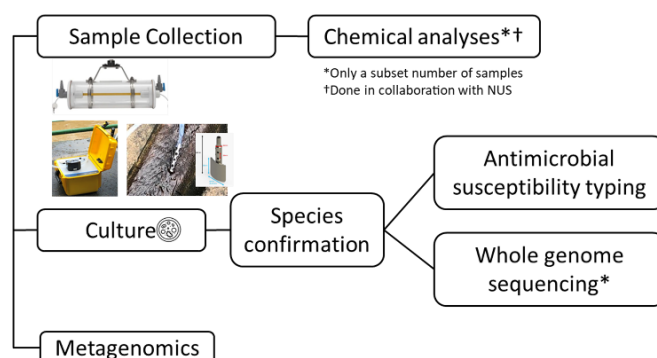
These findings showed that ESBL-Ec was not detected in beach water sampled from popular recreational beaches in Singapore. Of note, ESBL-Ec has been detected in previous studies by Zhong

et al.²³ and Ng et al.²² where water samples were collected from a freshwater reservoir and coastal sites used for aquaculture, respectively. The presence of ESBL-Ec in a freshwater reservoir and coastal seawater aquaculture has also been reported in other countries^{33 34}. While the current findings may suggest a low prevalence of ESBL-Ec, it should be noted that the study was conducted between Northeast Monsoon and inter-monsoon seasons in March 2021. As microbial compositions of surface water in the Singapore Strait may be impacted by seasonal variability³⁵, further sampling across different monsoon seasons will provide a better understanding of the prevalence of ESBL-Ec. Indeed, the global Tricycle project recommends environmental samples to be collected 6-8 times per sampling point³⁶. The collected data will be useful to understand not only their direct impacts to human and animal health, the potential risk of resistance determinants transferring to other microorganisms, but to also establish guides for mitigation measures.

Study 2: Antimicrobial resistant bacteria in coastal water and linked waterways

To understand the prevalence of AMR in coastal waters and waterways, water samples were collected from 28 coastal sites and 34 waterway sites, representing four different land-uses, namely agricultural, recreational, residential and industrial land-uses. Waterways refer to inland water streams that flow into the sea, and can be natural or man-made, such as rivers or canals, respectively. Conversely, coastal waters simply refer to seawater samples and are associated with waterways. Samples were collected from three different sampling periods, inter-monsoon (October 2021), Northeast monsoon (March 2022) and Southwest monsoon (August 2022) seasons. *E. coli*, *Klebsiella* spp., *P. aeruginosa* and *Enterococcus* spp. were enumerated according to the APHA standards.

Figure 59. Experimental design for AMR surveillance in waterways and coastal waters



³³ Rybak, B., N. Wawrzyniak, L. Wolska and M. Potrykus (2021). "Escherichia coli and Serratia fonticola ESBLs as a potential source of antibiotics resistance dissemination in the Tricity water reservoirs." Acta Biochim Pol 68: 437.

³⁴ Jeamsripong, S., V. Thaotumpitak, S. Anuntawirun, N. Roongrojmongkhon, E. R. Atwill and W. Hinthong (2022). "Molecular Epidemiology of Antimicrobial Resistance and Virulence Profiles of Escherichia coli, Salmonella spp., and Vibrio spp. Isolated from Coastal Seawater for Aquaculture." Antibiotics 11: 1688.

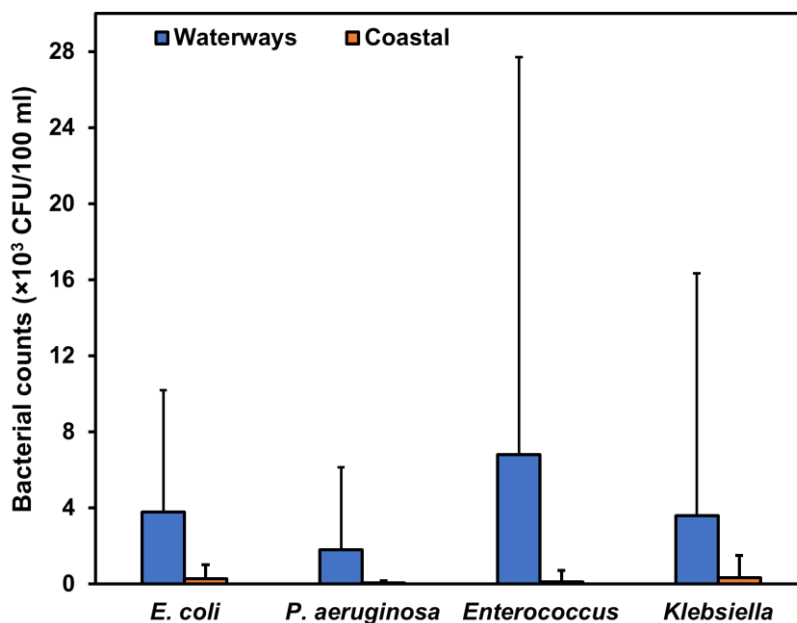
³⁵ Chénard, C., W. Wijaya, D. Vault, A. L. D. Santos, P. Martin, A. Kaur and F. M. Lauro (2019). "Temporal and spatial dynamics of Bacteria, Archaea and protists in equatorial coastal waters." Sci Rep 9(1): 16390.

³⁶ WHO (2021). WHO integrated global surveillance on ESBL-producing *E. coli* using a "One Health" approach: implementation and opportunities. <https://www.who.int/publications/i/item/who-integrated-global-surveillance-on-esbl-producing-e.-coli-using-a-one-health-approach>

Overall low bacterial counts in coastal water

As expected, lower bacterial counts were observed in coastal samples when compared to those from waterways. Levels of *E. coli* and *Enterococcus* spp. detected in coastal waters were also below the recommended guidelines of 500 CFU/100 ml by EU and 200 CFU/100 ml by WHO, respectively (Figure 60).

Figure 60. Mean bacterial counts across three different monsoon seasons



Prevalence of AMR differed across bacterial species

A subset of isolates from the four cited bacterial species of interest were also screened for the presence of AMR. A total of 242 ESBL-Ec isolates were isolated from the coastal and waterway samples, all of which showed high resistance to third-generation cephalosporins. At least 98% exhibited resistance to both cefotaxime and ceftriaxone, while 53% showed resistance to ceftazidime. Increasing resistance trends against tetracycline (63%), nalidixic acid (41%), sulfamethoxazole and trimethoprim (51%) and ciprofloxacin (41%) were also noted when antimicrobial susceptibility tests (AST) were performed. Multidrug resistance was similarly detected in *K. pneumoniae* with 32 out of 175 isolates (18.3%). Of note, both organisms exhibit increased resistance to drug classes from third-generation cephalosporins and beta-lactams, sulfonamides, fluoroquinolones and tetracyclines.

AST performed on *E. faecalis* isolates yielded approximately 20%, 11%, 5% and 4% resistance to tetracycline, erythromycin, chloramphenicol, and ciprofloxacin, respectively. AST on *E. faecium* isolates were still underway at the time of this report. Most *P. aeruginosa* isolates obtained were sensitive when tested against an antibiotic panel. Only 1 out of 239 isolates (0.4%) showed resistance to aztreonam.

Conclusions and Steps Forward

AMR surveillance in Singapore continued to be expanded in a stepwise fashion across the human, animal, food and environment sectors. In the human health sector, the national surveillance programme now includes private sector acute care hospitals and public sector primary care, which are being reported for the first time. Work is underway to collect AMR and AMU data from sentinel primary care clinics in the private sector.

Animal AMR surveillance has also expanded into two new areas: the free-roaming dog and wildlife populations. This helps to establish baselines for AMR in free-roaming dogs and wildlife, and potential antibiograms for sick companion animals in Singapore. Isolates from healthy free-roaming dogs were less frequently resistant than clinical isolates to antimicrobials tested, hence, healthy free-roaming dogs could serve as a proxy for healthy companion animals to understand baseline resistance levels. These findings underscore the need for continual monitoring and further investigation to better understand the AMR profiles of bacteria in companion and wild animals.

The environment is another area where global surveillance is limited. Studies in the environment are helping to build our understanding of AMR in Singapore's coastal areas and waterways. Although full phenotypic characterisation, determination of resistance determinants (resistomes and virulomes) and comparative genomics were still ongoing, the preliminary data reported here suggest prevalence of AMR in coastal water and waterway sites. It is therefore needful for regular AMR surveillance of these sites for risk assessment and mitigation where needed.

The next important step for surveillance is to establish an integrated One Health AMR surveillance system. A programme that collects harmonised data on priority organisms found in humans, animals, food and the environment in a manner that allows for integrated analysis, interpretation and risk assessment continues to be our goal. The One Health High Level Expert Panel (OHHLEP) recommends six steps for establishing One Health surveillance³⁷, which involves (1) developing the surveillance system scope; (2) identifying the data requirements; (3) developing the system design; (4) developing the system's governance; (5) developing integrated protocols; and (6) developing a joint implementation roadmap. A similar approach is taken: As we continue to strengthen sectoral surveillance capabilities and capacities, the governance and system structures to support such integration in Singapore are being developed in tandem. On-going research on potential transmission pathways will also guide the development of surveillance methodologies for priority targets of national relevance.

³⁷ OHHLEP, Developing One Health Surveillance Systems, One Health 17 (2023) 100617

Appendix I: AMU Methodology

General Information

I. Data on antimicrobial consumption

Antimicrobial consumption in humans

1. **Defined Daily Doses (DDD).** Hospitals report utilisation 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 WHO. The DDD is the assumed average maintenance dose per day for a medicine used for its main indication in adults. The DDD is a technical unit of use and does not necessarily reflect the recommended or average prescribed dose. The number of DDDs is calculated as follows:

$$\text{Number of DDDs} = \text{Total grams of active ingredient used} / \text{DDD value in grams}$$

The DDD value is assigned by the WHO only for drugs that already have an Anatomical Therapeutic Chemical (ATC) code. The ATC classification system is the most used method for aggregating data on medicines. Under this system, the active ingredients are classified into different groups according to the organ or system on which they act and their therapeutic, pharmacological and chemical properties.

2. Table I.1. Antimicrobials monitored by NARCC (as of 2021)

Grouping	Antimicrobial Agents (Year included for monitoring)
β Lactams and β-Lactamase Inhibitors (BLBLI)	Amoxicillin-clavulanate, IV Amoxicillin-clavulanate, oral (2019) Piperacillin-tazobactam, IV Ampicillin-sulbactam, IV and oral (2019) ³⁸
Novel BLBLI	Ceftolozane-tazobactam, IV (2019) Ceftazidime-avibactam, IV (2020)
Monobactam	Aztreonam, IV (2019)
Cephalosporins, 2nd gen	Cefoxitin, IV (2019)
Cephalosporins, 3rd & 4th gen	Cefixime, oral (2019) Cefoperazone, IV (2019) Cefotaxime, IV (2019) Ceftazidime, IV Ceftibuten, oral (2019) Ceftriaxone, IV Cefepime, IV
Cephalosporins, 5th gen	Ceftaroline, IV (2019)

³⁸ For oral, submitted as the whole strength of ampicillin-sulbactam (sultamicillin) tablet (375mg) as per WHO ATC (ref NASEP NOM 02/20, item 7.1 and NARCC NOM 02/20, item 4.2)

Carbapenems	Imipenem, IV Meropenem, IV Ertapenem, IV Doripenem, IV
Fluoroquinolones³⁹	Ciprofloxacin, IV and oral Levofloxacin, IV and oral Moxifloxacin, IV and oral
Polymyxins	Colistin, IV and nebulised use (combined) ⁴⁰ Polymyxin B, IV
MRSA/VRE agents	Teicoplanin, IV (2019) Vancomycin, IV and oral (combined) ⁴¹ Linezolid, IV and oral Tedizolid, IV and oral (2019) Daptomycin, IV
Tetracyclines	Doxycycline, IV and oral (2022) Eravacycline, IV (2022) Minocycline, IV and oral (2022) Tetracycline, oral (2022) Tigecycline, IV
Lincosamides	Clindamycin, IV and oral (2019)
Others	Fosfomycin, IV and oral (2019)
Antifungals	Fluconazole, IV and oral Voriconazole, IV and oral Posaconazole, oral Isavuconazole, IV and oral (2020) Caspofungin, IV Anidulafungin, IV Micafungin, IV (2019) Amphotericin B conventional Amphotericin B liposomal Itraconazole, IV and oral

3. **Data limitations.** (1) *Paediatric use* - DDDs are normally assigned based on use in adults. For medical products approved for use in children, the dose recommendations will differ based on age and weight. DDDs are therefore not ideal for estimating drug utilisation in children. However, for NARCC's purposes, DDD has been applied to both adult and paediatric use. (2) *DDD changes* - DDD is calculated based on prevailing values published by WHO. WHO regularly reviews and updates DDDs because dosages may change over time e.g. due to introduction of new main indications. These changes should be taken into consideration when interpreting AMU trends presented in DDD. (3) *The use of inpatient days as a denominator* allows for a weightage of overall utilisation to be obtained and allows normalisation across hospitals of

³⁹ For levofloxacin and moxifloxacin, submitted separately according to the route of administration (IV and oral) w.e.f. 2022 (ref NASEP NOM 03/22, item 3.1 and NARCC NOM 03/22, item 4.1)

⁴⁰ Submitted as a combined total as some hospitals are unable to differentiate between IV and nebulised use of colistin (ref NARCC NOM 02/20, item 4)

⁴¹ Submitted as a combined total as some hospitals are unable to differentiate between IV and oral use of vancomycin w.e.f. 2022 (ref NARCC NOM 02/20, item 4)

different sizes. However, antimicrobial utilisation includes both inpatient and outpatient sources. Nevertheless, consistent application of the same methodology allows for year-on-year trending.

Antimicrobial consumption in animals

1. Sales data serving as national consumption estimates for the animal sector through are obtained a voluntary survey sent to veterinary drug wholesalers annually. Wholesalers are requested to provide information on the following:
 - Name of antimicrobial product
 - Strength
 - Unit
 - Active ingredient(s)
 - Route of administration
 - End-user the product is supplied to (e.g., Vet clinic, land farm, fish farm etc)
 - Purpose (therapeutic use or growth promotion)
 - Quantity supplied

Quantities (in kg) of the antimicrobials are calculated from the sales data provided and grouped into respective antimicrobial classes, stratified by type of use (veterinary medical use or growth promotion), species group (e.g., food-producing animals and non-food producing animals) and route of administration.

2. Data limitations

- (1) Given the voluntary nature of this survey, not all veterinary drug wholesalers engaged by NParks participated in the survey. Therefore, the data obtained may be an underestimate of the actual number of antimicrobials supplied to and utilised by the animal sector.
- (2) As antimicrobial sales data only serves as a proxy of antimicrobial utilisation in animals, it is insufficient to illuminate consumption patterns and volumes at the level of the end-user (vet clinics, farms). Sales trends should also be interpreted with caution as they do not necessarily correspond with utilisation. Therefore, the collection of comprehensive antimicrobial utilisation data at the level of the end-user remains the most valuable method to determine utilisation trends and guide the development of targeted and effective strategies and interventions.

Appendix II. AMR Methodology

Collection, Identification and susceptibility testing of bacterial isolates

From human specimens

1. **Data collection.** Clinical isolates are counted once in every six-month period per patient. Duplicate isolates from the same patient, sample type and bacterial species collected within each six-month period are excluded. While clinical isolates may include colonisation, they provide a useful indicator for the total AMR burden, which in turn impacts the consumption of hospital resources (e.g., isolation rooms, gowns, gloves and manpower). Bacteraemia rates generally represent true infection. Screening samples are excluded in most instances.
2. **List of priority pathogen-drug combinations and sample types** for surveillance (Table II.1).

Table II.1. NARCC priority pathogens for surveillance

Pathogen	Specific resistance	Specimen types to report
<i>Staphylococcus aureus</i>	Cloxacillin (or equivalent anti-staphylococcal penicillin), vancomycin	(i) All clinical specimens (ii) Blood (for MRSA only)
<i>Escherichia coli</i>	Ceftriaxone (or equivalent 3 rd -generation cephalosporin), ciprofloxacin, carbapenem (meropenem or imipenem)	(i) All clinical specimens (ii) Blood
<i>Klebsiella pneumoniae</i>	Ceftriaxone (or equivalent 3 rd -generation cephalosporin), ciprofloxacin, carbapenem (meropenem or imipenem)	(i) All clinical specimens (ii) Blood
<i>Pseudomonas aeruginosa</i>	Carbapenem (meropenem or imipenem)	(i) All clinical specimens (ii) Blood
<i>Acinetobacter baumannii</i>	Carbapenem (meropenem or imipenem), MDR ^(a)	(i) All clinical specimens (ii) Blood (for carbapenem resistance only)
<i>Enterobacterales</i>	Carbapenemase-producing	(i) All clinical specimens (ii) Screening specimens
<i>Enterococcus faecalis</i> , <i>Enterococcus faecium</i>	Vancomycin	(i) All clinical specimens (ii) Blood
<i>Clostridioides difficile</i>	-	(i) All clinical specimens (stool)
<i>Candida auris</i>	-	(i) All clinical specimens (ii) Screening specimens ^(b) (iii) Blood

3. **Standards and AST methods.** Clinical isolates are tested for antimicrobial susceptibility by hospitals' clinical microbiology laboratories in accordance with the standards of Clinical & Laboratory Standards Institute (CLSI), European Committee on Antimicrobial Susceptibility Testing (EUCAST), or with the Calibrated Dichotomous Sensitivity (CDS, Australia) method,

where applicable. Laboratory and AST methods are determined by hospital laboratories, and include disk diffusion, e-test and MIC methods where appropriate.

4. **Metrics.** The data are presented as (i) incidence density per 10,000 inpatient days and where relevant, (ii) the proportion (%) of resistant clinical isolates. The resistance percentage is typically calculated only when the denominator contains at least 30 isolates to ensure a minimum level of precision in the calculation. For NARCC's purposes, resistant isolates include those of intermediate susceptibility.
5. **Data limitations.** The use of inpatient days as a denominator allows for a measurement of the incidence density (cases per 10,000 inpatient days) to be obtained and allows normalisation across hospitals of different sizes. However, antimicrobial resistance data are obtained from laboratory detection from samples submitted, which may include outpatient sources. Nevertheless, consistent application of the same methodology will allow year-on-year observation of trends.

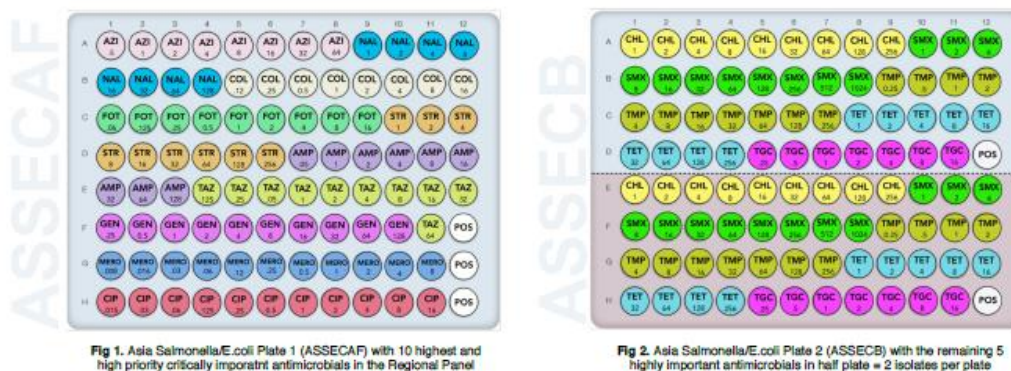
From animal specimens

1. **Data collection.** Samples collected from the respective AMR surveillance programmes were obtained from veterinarians from NParks or veterinary clinics/hospitals (Table II.2). These samples were then sent to the CAVS for testing. Subsequently, the AST results were compiled and analyzed by the NParks AMR workgroup at least once every six months.

Table II.2. Sampling matrix for the respective AMR surveillance in companion animals and wildlife

Surveillance	Bacteria	Sample Type	Frequency
Free-roaming dogs (active)	(i) <i>E. coli</i> , <i>K. pneumoniae</i> (ii) MRSA/MRSP	(i) Faecal or rectal swabs (ii) Ear swabs	Monthly
Sick companion animals (passive)	(i) <i>E. coli</i> , <i>K. pneumoniae</i> (ii) MRSA/MRSP	Various sample types (clinical samples)	Ad-hoc
Wildlife (passive)	<i>E. coli</i>	(i) Faecal or rectal swabs (ii) Organs (dead animals)	(i) Monthly (ii) Ad-hoc

2. **Standards & AST methods.** AST were performed on all *E. coli* and *K. pneumoniae* by broth microdilution using the Sensititre ASSECAF and ASSECB plates (Figure II.1). The method and breakpoint interpretations were in accordance with the CLSI and/or EUCAST standards, where applicable. The Sensititre plates contained 15 veterinary important antimicrobials (ampicillin, azithromycin, ceftazidime, cefotaxime, chloramphenicol, ciprofloxacin, colistin, gentamicin, meropenem, nalidixic acid, sulfamethoxazole, streptomycin, tigecycline, tetracycline, and trimethoprim) from 10 antimicrobial classes.

Figure II.1. Sensititre™ Asia Surveillance Plates ASSECAF (left) and ASSECB (right).⁴²

AST were performed on *S. aureus* and *S. pseudintermedius* using VITEK AST GP80 cards. The method and breakpoint interpretations were in accordance with the CLSI and/or EUCAST standards, where applicable. The VITEK GP80 card contained 19 antimicrobials (amoxicillin/clavulanic acid, cefalotin, ceftiofur, chloramphenicol, clindamycin, doxycycline, enrofloxacin, erythromycin, gentamicin, kanamycin, marbofloxacin, nitrofurantoin, neomycin, penicillin, pradofloxacin, oxacillin, tetracycline and trimethoprim/sulfamethoxazole) from 11 antimicrobial classes that are of veterinary importance to companion animals. The isolates were tested for methicillin resistance and specific resistance genes, in particular, the *mecA* gene, which is the most common gene conferring methicillin resistance in staphylococci. Cefoxitin-resistant *S. aureus* carrying the *mecA* gene are identified as MRSA, while oxacillin-resistant *S. pseudintermedius* with the *mecA* gene are identified as MRSP.

3. **Metrics.** The data were presented as (i) prevalence or proportion of samples, (ii) the proportion (%) of MDR bacteria, and (iii) the percentage (%) of resistant isolates. MDR is defined as being resistant to three or more classes of antimicrobials. Resistant isolates did not include those of intermediate susceptibility.

From farms

1. **Data collection.** Samples collected from local farm AMR surveillance were obtained from inspectors from SFA (Table II.3). The testing of *Salmonella* spp. and *E. coli* in the poultry and ruminant farms were under the purview of NParks until Jun 2021. In July 2021, the testing of *Salmonella* spp. in the poultry farms was taken over by SFA. The testing of *Salmonella* spp. and *E. coli* in the ruminant farms and the testing of *E. coli* in poultry farms remained under the purview of NParks. Subsequently, the AST results were compiled and analyzed by the NParks AMR workgroup at least once every six months. The AST results from *Salmonella* in the poultry farms were jointly analysed by the NParks and SFA AMR workgroup.

⁴² FAO (2019). Towards transforming AMR surveillance capacities in food and agriculture in Asia, Vol 2. No. 1 (Project OSRO/RAS/502/USA)

Table II.3. Sampling matrix for AMR surveillance in local farms

Surveillance	Bacteria	Sample Type	Frequency
Poultry (chicken and quail) farms	(i) <i>Salmonella</i> spp.	[Jan 2020 – Jun 2021] Various sample types, including environmental swabs, pooled organs, faeces	Quarterly
	(ii) <i>E. coli</i>	[Jul 2021 onwards] Pooled drag swabs	
Ruminant (cattle and goat) farms	(i) <i>Salmonella</i> spp. (ii) <i>E. coli</i>	Pooled fecal samples	Quarterly

- Standards & AST methods.** AST were performed on all *Salmonella* spp. and *E. coli* by broth microdilution using the Sensititre ASSECAF and ASSECB plates (Figure II.1). The method and breakpoint interpretations were in accordance with the CLSI and/or EUCAST standards, where applicable. The Sensititre plates contained 15 veterinary important antimicrobials from 10 antimicrobial classes.
- Metrics (where applicable).** The data were presented as (i) prevalence or proportion of samples, (ii) the proportion (%) of MDR bacteria, and (iii) the percentage (%) of resistant isolates. MDR is defined as being resistant to three or more classes of antimicrobials. Resistant isolates did not include those of intermediate susceptibility.

From food samples

- Data collection.** SFA's AMR surveillance adopts the FAO's Regional AMR Monitoring and Surveillance Guidelines to ensure a standardised and harmonised protocol for AMR monitoring. This harmonisation also ensures comparability of AMR data to maximise potential value of findings at regional level in future.
- Target organisms and samples.** SFA monitors resistance to antimicrobial agents in commensal bacteria and food-borne pathogens from target food and food-producing animals intended for consumption and monitors trends of AMR bacteria (*E. coli* and *Salmonella* spp.) in relevant imported and retail food products, animal feed, food producing animals, as well as farm and food processing environments.
- Standards and AST methods.** *Salmonella* spp. and *E. coli* bacteria from food sources, establishments, and retail samples were isolated and tested by SFA according to ISO17025 accredited methods and international standards for microbiological identification and antibiotic susceptibility testing. SFA adopts the CLSI M100 interpretive breakpoints for determining antibiotic susceptibility.

Tests for MIC were performed with 10 classes of 11 antimicrobials (ampicillin, cefotaxime, ceftazidime, ciprofloxacin, chloramphenicol, colistin, gentamicin, meropenem, sulfamethoxazole, tetracycline and trimethoprim) using the Sensititre™ Asia Surveillance Plates for *Salmonella/E. coli*.

The Combination Disk Diffusion test (CDT) is based on the capacity of clavulanic acid to inhibit ESBL, and the synergy that is produced in combination with cefotaxime and the combination

with ceftazidime. The interpretation of the results is based on the zone size of each cephalosporin alone cefotaxime (CTX), 30µg and ceftazidime (CAZ), 30µg, compared with the discs containing the combination of cephalosporin and clavulanic acid, (Cefotaxime-clavulanic acid (CTX/CA) and Ceftazidime- clavulanic acid (CAZ/CA), 40 ug (30µg/10µg) each. If the zone diameter of the disc with the combination for any or both cephalosporin is higher or equal to 5mm, the result is interpreted as positive for ESBL.

VITEK® 2 AST-GN79 ESBL Test was used for confirmation of the ESBL phenotype in 2020 and 2021. It is a confirmatory test for those ESBLs inhibited by clavulanic acid, and it utilizes cefepime, cefotaxime, and ceftazidime, with and without clavulanic acid, to determine a positive or negative result.

Antimicrobial susceptibility testing is carried out by SFA using the Sensititre™ system to determine minimum inhibitory concentration (MIC) in accordance with accredited methodology, adopting FAO's Asia Surveillance plates for *Salmonella*/*E. coli* (ASSEc) Panel.

The Asia Surveillance plates for *Salmonella*/*E. coli* (ASSEc) were designed by FAO in collaboration with Singapore to provide an antibiotic panel consistent with recommendations in the FAO Regional AMR surveillance Guideline #1 (AMR monitoring and surveillance in bacteria from healthy food animals intended for consumption). The ASSEc plates consist of a range of antibiotic dilutions covering the clinical breakpoints, ECOFFs and recommended ranges appropriate for the prescribed bacterial strain. Plate 1 (ASSECAF) consists of the 10 highest priority critically important antimicrobials in the Regional Panel. Plate 2 (ASSECB) consists of the next five highly important antimicrobials in half plate format, such that isolates could be tested per plate. The results are interpreted based on CLSI breakpoints (M100, 30th Edition) and (EUCAST Ver 11.0, 2021) to determine the Minimum Inhibitory Concentration (MIC).

a. ASSECAF Asia *Salmonella*/ *E. coli* Sensititre Plate 1

Antimicrobial panel and interpretive criteria for target bacteria adapted according to FAO (2019).

Antibiotic Panel and Dilution Range			Breakpoints			
No	Antibiotic	Concentration (µg/ml)	S	I	R	Interpretative guideline
1	Azithromycin	0.5-64	≤ 16	-	≥ 32	CLSI M100 30th Edition
2	Nalidixic Acid	1-128	≤ 16	-	≥ 32	
3	Colistin	0.12-16	-	≤ 2	≥ 4	
4	Cefotaxime	0.06-16	≤ 1	2	≥ 4	
5	Streptomycin	1-256	-	-	-	CLSI M100 30th Edition
6	Ampicillin	0.05-128	≤ 8	16	≥ 32	
7	Ceftazidime	0.125-64	≤ 4	8	≥ 16	
8	Gentamicin	0.25-128	≤ 4	8	≥ 16	
9	Meropenem	0.008-8	≤ 1	2	≥ 4	
10	Ciprofloxacin (<i>E. coli</i>)	0.015-16	≤ 0.25	0.5	≥ 1	
	Ciprofloxacin (<i>Salmonella</i>)	0.015-16	≤ 0.06	0.12-0.5	≥ 1	

b. **ASSECAF Asia *Salmonella*/ *E. coli* Sensititre Plate 2**

Antibiotic Panel and Dilution range			Breakpoints			
No	Antibiotic	Concentration (µg/ml)	S	I	R	Interpretative guideline
1	Chloramphenicol	1-256	≤ 8	16	≥ 32	CLSI M100 30th Edition
2	Sulfamethoxazole	1-1024	≤ 256	-	≥ 512	
3	Trimethoprim	0.25-256	≤ 8	-	≥ 16	
4	Tetracycline	1-256	≤ 4	8	≥ 16	
5	Tigecycline	0.25-16	≤ 0.5	-	> 0.5	EUCAST Ver 11.0, 2021

4. **Metrics.** Resistance data are presented as proportion of resistant isolates (%R), which is the number of isolates resistant to the specified antimicrobial as a proportion of all isolates tested. For SFA's monitoring, resistant isolates exclude those of intermediate susceptibility.
5. **Data limitations.** Rates of detection in food imports or along the food supply chain do not necessarily correlate with prevalence. Sampling of food products is primarily for food safety purposes and are therefore subject to risk-based sampling. For instance, higher-risk products may be subject to increased sampling. Food sources and product types also vary from year to year. These factors should be taken into consideration when interpreting data and trends.