Retrospective analysis of antimicrobial resistance in bacterial pathogens from pet rabbits in Hong Kong, 2019–2022

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Abstract. Antimicrobial resistance (AMR) is a growing threat to human and animal health. Data are limited on the prevalence of resistant bacteria in pet rabbits. Therefore, we aimed to identify prevalent bacterial infections and AMR profiles among pet rabbits in Hong Kong (HK). Our search of the CityU Veterinary Diagnostic Laboratory (VDL) database found 301 cases of pet rabbits submitted for bacteriologic and antimicrobial susceptibility testing by veterinarians at 20 exotic veterinary clinics across HK between 2019 and 2022. The rabbits were of 8 different breeds and had a median age of 6.5 y, with 54.8% males, 40.2% females, and 5% unspecified. Of the 301 samples received, 168 (55.8%) had positive bacterial growth; 125 (74.4%) had single bacterial isolates, and 43 (25.6%) had mixed cultures. Cultures included *Enterococcus faecalis* (21.3%) as the most frequently isolated gram-positive bacterium, followed by *Streptococcus intermedius* (12.5%), and *Staphylococcus aureus* (11.3%). The most frequently isolated gram-negative bacteria were *Pseudomonas aeruginosa* (18.1%), followed by *Escherichia coli* (8.3%), *Pasteurella multocida* (6.9%), and *Klebsiella pneumoniae* (4.2%). Approximately 83% of the isolates had acquired resistance to at least one antimicrobial agent, and 49.4% were multidrug-resistant. The isolated bacteria had high levels of resistance to penicillin (69.8%), clindamycin (47.4%), and doxycycline (46.9%). Our findings highlight the high levels of AMR in bacteria isolated from pet rabbit clinical samples in HK; many of these bacteria are zoonotic and pose a public health threat.

Keywords: antimicrobial resistance; bacterial pathogens; clinical samples; rabbits; Hong Kong.

Antimicrobial resistance (AMR) is a recognized global public health threat that occurs when microorganisms become unresponsive to antimicrobials.[26] Resistance to antimicrobials may be either intrinsic (natural resistance) or acquired (due to mutations in pre-existing or previously acquired genes or horizontal gene transfer).[2] The excessive use of antimicrobials significantly contributes to the emergence, development, and spread of AMR bacteria. The growing prevalence of AMR among various bacterial strains, including *Pasteurella multocida*,[13] *Staphylococcus aureus*,[3] *Escherichia coli*,[31] *Enterococcus* spp.,[20] and others, is a serious health concern,[27] with severe consequences for bacterial infections in animals and humans. Consequently, AMR has become a growing problem that poses a threat to society, animal health, and the global environment.

Extensive research and surveillance on AMR in animals are currently conducted worldwide, with a particular focus on food-producing animals, such as chickens, cattle, and pigs.[20] These species receive significant attention from academia given their role in the epidemiology of AMR[1]; resistant organisms can be transmitted directly from animals to foods of animal origin, farm workers, the feeding environment,[10] as well as the surrounding land and crops.[1] The widespread use of antimicrobials in veterinary medicine has contributed to an increase in AMR bacteria, which has implications for both food animals and pets.[23] These AMR bacteria, including *Salmonella*, *Campylobacter*, *S. intermedius*, and *E. coli*,[15]

pose particular concerns given their potential for transmission from pets to humans. Moreover, infections caused by AMR bacteria tend to result in more severe illnesses, prolonged hospitalization, and increased healthcare costs.[34]

In Hong Kong (HK), AMR bacteria are either highly prevalent or rapidly increasing, with limited, less effective, and more expensive treatment options.[5] Despite the significant concern expressed by the HK government and academic community about AMR bacteria from food and companion animals, little attention has been given to AMR in pet rabbits. Pet rabbits serve as potential sources of zoonotic AMR bacteria, highlighting the importance of practicing proper hygiene and healthcare measures when interacting with these pets to prevent disease transmission to humans.[36] In HK, rabbits constitute 2.3% of the pet population, with dogs and cats accounting for 37.7% and 18.9%, respectively.[14]

To our knowledge, there is limited information regarding the prevalence of AMR bacteria in the pet rabbit population,[25]

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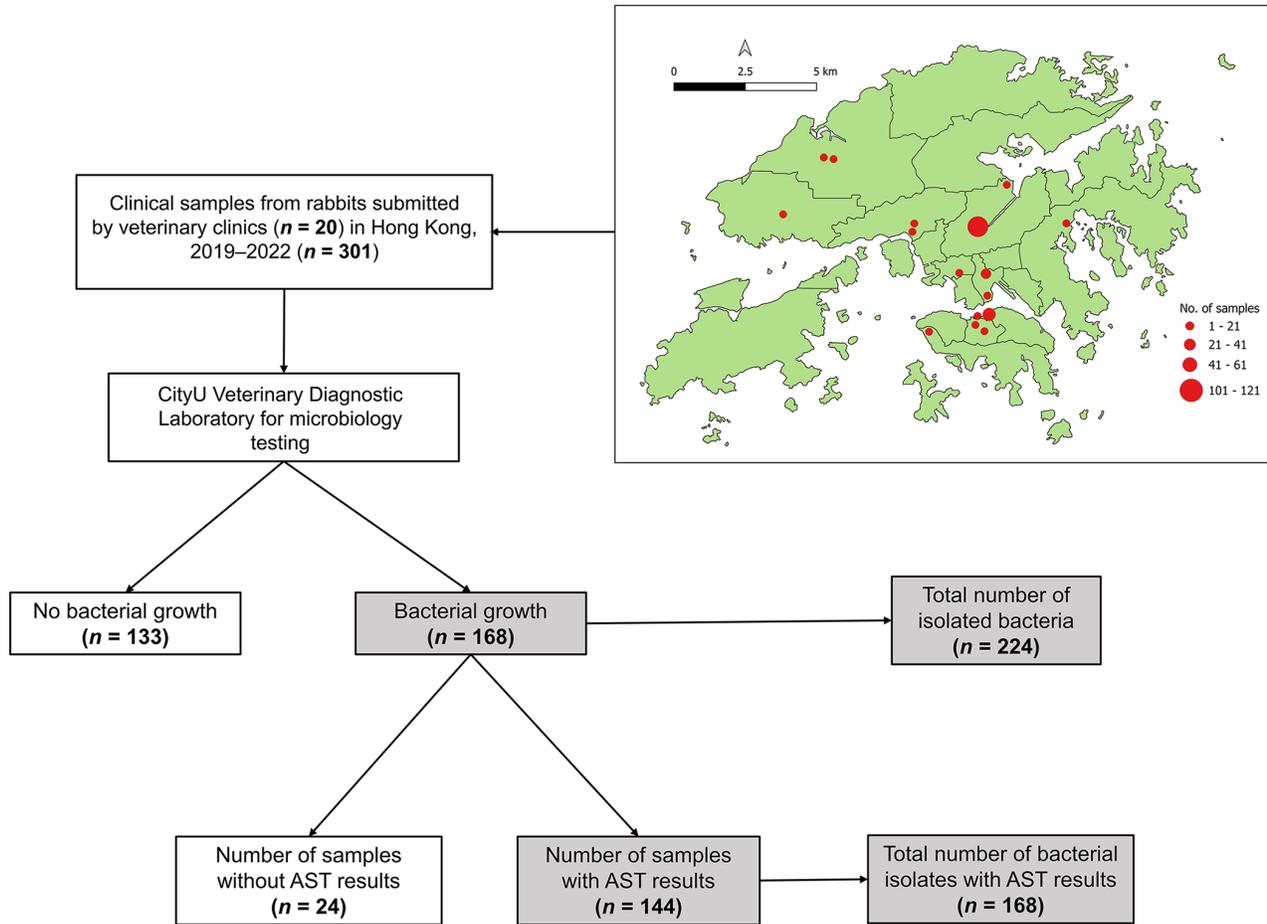
Figure 1. A schematic diagram illustrates the distribution map of veterinary clinics, along with a flowchart describing data extraction.

especially in HK. Therefore, we aimed to fill this knowledge gap by identifying the most prevalent bacterial infections and AMR profiles among clinical samples from pet rabbits in HK. Ultimately, a better understanding of the distribution and AMR profiles of bacterial strains in pet rabbits may lead to the development of a more effective antimicrobial stewardship program.

Materials and methods

Data collection and management

We searched the electronic medical record database of CityU Veterinary Diagnostic Laboratory (VDL; City University of Hong Kong, Hong Kong SAR, China) for rabbit clinical samples submitted by veterinarians at exotic veterinary clinics across HK (Fig. 1) from March 2019 to December 2022.

We included rabbit clinical samples submitted specifically for microbiologic and/or antimicrobial susceptibility testing (AST). We downloaded 304 electronic diagnostic testing records. We excluded 3 rabbit clinical samples submitted for fungal culture. We extracted relevant data from each record, including the veterinary clinic's name, rabbit breed, sex, date of birth, sample type, sampling site, sampling date, bacteriologic examination results, and AMR profiles.

Bacteriologic examination

Rabbit clinical samples, such as fluids (mostly urine and pus), swabs (mostly wound and abscess swabs), tissue, and fecal samples, were processed for microbiologic culture and identification following standard operating procedures. All samples were cultured on appropriate media for aerobic and anaerobic testing. Bacterial colonies were collected, and bacteria were identified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker) and analyzed with the MALDI Biotyper (Bruker) software.

Antimicrobial susceptibility testing

A bacterial suspension was prepared from pure cultures of growing bacteria and subjected to either the disc diffusion or broth microdilution techniques for AST. The antimicrobial panel utilized varied based on the bacterial species; the panel consisted of various of the following 30 antimicrobials: penicillin (amoxicillin–clavulanic acid, ampicillin, penicillin, ticarcillin–clavulanate), aminoglycoside (amikacin, gentamicin, tobramycin, framycetin, neomycin), macrolides (azithromycin), cephalosporins (cefazolin, cefixime, cefoxitin, cefpodoxime, ceftazidime, ceftriaxone, cefuroxime axetil,

Table 1. Characteristics of Hong Kong rabbit samples, stratified by sampling year.

Characteristic	2019, n (%)	2020, n (%)	2021, n (%)	2022, n (%)	Total, n (%)
Sample	26 (8.7)	94 (31.2)	84 (27.9)	97 (32.2)	301 (100.0)
Sample type					
Fluid (mainly urine)	11 (19.0)	16 (27.6)	11 (19.0)	20 (34.4)	58 (19.3)
Swab	15 (6.5)	71 (30.7)	70 (30.3)	75 (32.5)	231 (76.7)
Tissue	0	5 (50.0)	3 (30.0)	2 (20.0)	10 (3.3)
Feces	0	2 (100)	0	0	2 (0.7)
Sex					
Female	11 (9.1)	45 (37.2)	27 (22.3)	38 (31.4)	121 (40.2)
Male	15 (9.1)	48 (29.1)	46 (27.9)	56 (33.9)	165 (54.8)
Unknown	0	1 (6.7)	11 (73.3)	3 (20.0)	15 (5.0)
Age, y					
Median (IQR)	8.7 (6.1–10.8)	6.4 (3.1–8.7)	5.8 (1.8–8.9)	6.8 (2.9–9.3)	6.5 (3.0–9.1)
Breed					
Angora	4 (57.1)	0	1 (14.3)	2 (28.6)	7 (2.3)
Dutch	0	1 (12.5)	2 (25.0)	5 (62.5)	8 (2.7)
Dwarf	1 (3.2)	2 (6.5)	5 (16.1)	23 (74.2)	31 (10.3)
English spot	0	2 (100)	0	0	2 (0.7)
Lionhead	4 (11.4)	11 (31.4)	7 (20.0)	13 (37.1)	35 (11.6)
Lop (Mini, French, Holland)	4 (8.7)	8 (17.4)	16 (34.8)	18 (39.1)	46 (15.3)
New Zealand white	0	0	5 (62.5)	3 (37.5)	8 (2.7)
Rex	0	1 (50.0)	1 (50.0)	0	2 (0.7)
Unknown	13 (8.0)	69 (42.6)	47 (29.0)	33 (20.4)	162 (53.2)
Bacterial infection					
Yes	10 (6.0)	49 (29.2)	52 (30.9)	57 (33.9)	168 (55.8)
No	16 (12.0)	45 (33.8)	32 (24.1)	40 (30.1)	133 (44.2)

IQR = interquartile range.

cephalexin), fluoroquinolones (ciprofloxacin, enrofloxacin, ofloxacin, orbifloxacin), tetracyclines (doxycycline), florfenicol, clindamycin, marbofloxacin, cefovecin, chloramphenicol, trimethoprim–sulfamethoxazole, and fusidic acid.

Results were interpreted as sensitive or resistant based on the Clinical and Laboratory Standard Institute (CLSI) guidelines.[6] Furthermore, intrinsic resistance to specific antimicrobials was excluded from data analysis, following the latest EUCAST expert rules on intrinsic resistance and exceptional phenotypes.[11] Only acquired resistance was analyzed and presented. A variety of control strains, such as *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Streptococcus pneumoniae* ATCC 49619, were used for routine internal quality control testing.

The multiple antibiotic resistance (MAR) index was calculated by dividing the number of antimicrobials to which bacterial isolates showed resistance by the total number of antimicrobials tested.[18] Multidrug resistance (MDR) was defined as the resistance of an isolate to ≥1 agent in ≥3 antimicrobial classes.[24]

Data analysis

The extracted data were entered into Microsoft Excel and subsequently imported into R software (v.4.2.0, https://www.r-project.org/) for data analysis and visualization. Categorical variables were presented as numbers and percentages; continuous variables were reported as median and interquartile range (IQR). To compare the MAR index of isolates from different years, a one-way

ANOVA was performed. A $p \leq 0.05$ was considered statistically significant. QGIS software (v.3.30.3) was used to create a map of the approximate locations of veterinary clinics.[29]

Results

Study population

Between March 2019 and November 2022, CityU VDL received 301 clinical samples from rabbits. Veterinarians from 20 clinics in HK (Fig. 1) collected and submitted these samples for bacteriologic testing and AST.

The rabbits were 3–9.1-y-old, with a median age of 6.5 y. Among the sampled rabbits, 54.8% were males, 40.2% females, and sex information was not available for the remaining 5% (Table 1). The breed of rabbits was not recorded for >50% of the rabbits. However, for the remaining 50%, samples originated from 8 different breeds, with the highest representation from the Lop breed (15.3%), followed by Lionhead (11.6%), and Dwarf (10.3%). Samples consisted primarily of swabs (76.7%), predominantly wound and abscess swabs, followed by fluid samples (19.3%), mainly urine samples (Table 1).

Microbiologic results

Of the 301 samples, bacterial growth was reported in 168 (55.8%) samples; 125 (74.4%) had single bacterial growth, and 43 (25.6%) had mixed bacterial growth. A total of 35 bacterial species were isolated from the samples (Fig. 2),
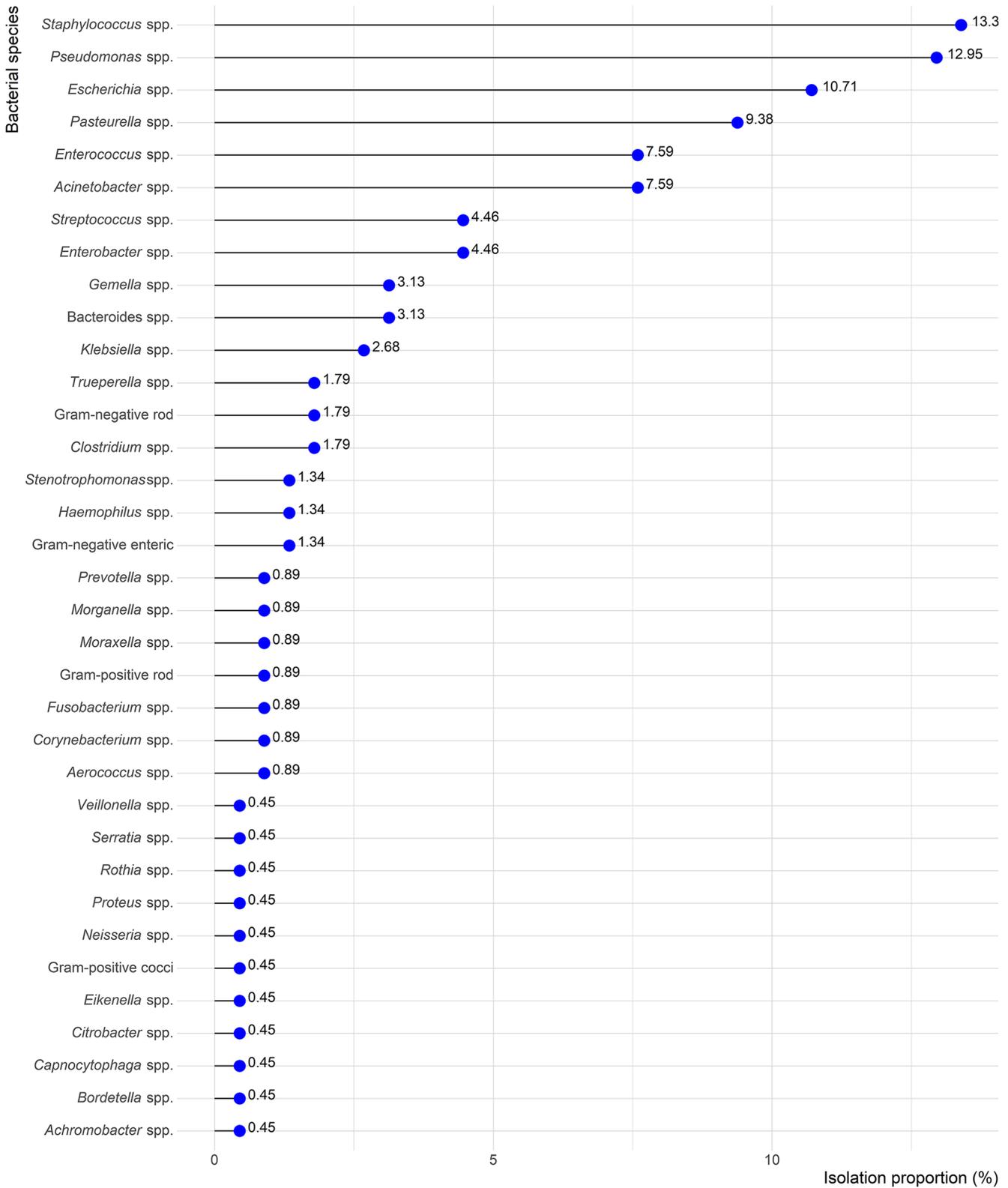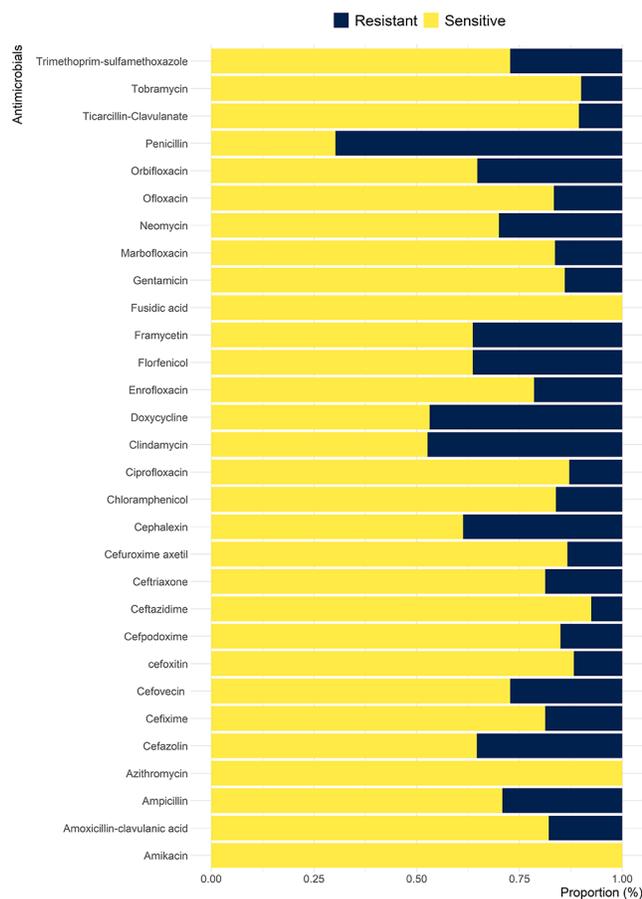
Figure 2. Distribution of the bacterial isolates recovered from 168 rabbit clinical samples in Hong Kong.

Table 2. Distribution of bacterial groups recovered from 168 rabbit clinical samples in Hong Kong, stratified by sampling year.

Bacterial spp.	2019, n (%)	2020, n (%)	2021, n (%)	2022, n (%)	Total, n (%)
Gram-negative	5 (3.5)	40 (27.8)	54 (37.5)	45 (31.3)	144 (64.3)
Gram-positive	5 (6.3)	21 (26.3)	24 (30.0)	30 (37.5)	80 (35.7)
Total	10 (4.5)	61 (27.2)	78 (34.8)	75 (33.5)	224 (100.0)

Figure 3. Frequency of antimicrobial resistance among bacterial isolates recovered from 168 rabbit clinical samples in Hong Kong.

with gram-negative bacteria the most identified species (Table 2). Among these 35 identified bacterial species, *Staphylococcus* spp. was the most frequently isolated; 54% of the *Staphylococcus* isolates were identified as *S. aureus*. Within the gram-negative bacteria, the most represented were *P. aeruginosa* (18.1%), *E. coli* (8.3%), *P. multocida* (6.9%), and *K. pneumoniae* (4.2%). *E. faecalis* (21.3%), *S. intermedius* (12.5%), and *S. aureus* (11.3%) were the most commonly identified gram-positive bacteria (Table 2).

Antimicrobial susceptibility test

The AST revealed that 82.7% of the isolates recovered from clinical rabbit samples were resistant to ≥1 antimicrobial

(Fig. 3). Most of the isolates had high resistance to penicillin (69.8%), clindamycin (47.4%), and doxycycline (46.9%). However, all isolates were susceptible to fusidic acid, azithromycin, and amikacin.

Most (66.7%) of *S. aureus* isolates were resistant to 1 antimicrobial, and the remaining 33.3% were resistant to 2 antimicrobials (Table 3). Conversely, 50% of *E. coli* isolates were resistant to >3 antimicrobials.

MDR was observed in 49.4% of bacterial isolates from rabbit clinical samples; *E. coli*, *E. faecalis*, *P. aeruginosa*, and *P. multocida* had the highest levels of MDR among all isolates. The average MAR index was 0.33, ranging from 0.05 to 1.00 (Fig. 4). The difference in the average MAR index between gram-positive and -negative bacterial isolates was significant ($p = 0.001$; Fig. 4A). However, we found no significant difference ($p = 0.172$) in the average MAR index among isolates from each year. In addition, differences were significant in the MAR index between bacterial species, such as *Staphylococcus* spp. versus *Pseudomonas* spp. ($p = 0.002$) and *Staphylococcus* spp. versus *Haemophilus* spp. ($p = 0.001$; Fig. 4B).

Discussion

We found that 55.8% of 301 samples from pet rabbits had bacterial growth, with *Staphylococcus* spp. (13.39%) and *Pseudomonas* spp. (12.95%) being the most frequently isolated species. The frequency of gram-negative bacterial species (64.3%) exceeded that of gram-positive species (35.7%). The predominant gram-positive species in our study were *Staphylococcus* spp. and *Enterococcus* spp., consistent with previous reports in both farmed[21] and pet[28] rabbits. A study on pet rabbits in Tunisia indicated that 69% and 73% of fecal samples contained *E. coli* and enterococci, respectively.[4] However, isolation rates of *E. coli* (57%) and enterococci (83%) differed in pet rabbits from Portugal.[31] These variations may be attributed to differences in the epidemiologic and ecologic characteristics of the study populations as well as the sample size.

E. faecalis was the most frequently identified gram-positive bacterium (21.3%) in rabbit clinical samples in HK. A Spanish study found that *E. faecalis* represented ~1.3% of bacterial isolates, whereas other *Enterococcus* spp. comprised just 1.23%.[12] This disparity in isolation rates may be explained by the substantial number of clinical samples sourced from urine in our study, especially considering that *E. faecalis*, a gram-positive bacterium, is commonly associated with urinary infections.[17] The small proportion of *E. coli*, *P. multocida*, and *K. pneumoniae* isolates found in

Table 3. Distribution of antimicrobial resistance in common bacterial isolates recovered from 168 rabbit clinical samples in Hong Kong.

Bacterial isolate	Total, n	Resistant to no. of antimicrobials				MDR, n (%)	Average MAR index (range)
		1, n (%)	2, n (%)	3, n (%)	>3, n (%)		
Actinobacillus suis	5	3 (60.0)	1 (20.0)	0	1 (20.0)	1 (20.0)	0.17 (0.07–0.40)
Streptococcus intermedius	5	3 (60.0)	1 (20.0)	1 (20.0)	0	1 (20.0)	0.15 (0.09–0.30)
Klebsiella pneumoniae	6	2 (33.3)	2 (33.3)	0	2 (33.3)	2 (33.3)	0.41 (0.09–1.00)
Staphylococcus aureus	9	6 (66.7)	3 (33.3)	0	0	0	0.11 (0.06–0.18)
Pasteurella multocida	9	1 (11.1)	3 (33.3)	2 (22.2)	3 (33.3)	5 (55.6)	0.25 (0.09–0.50)
Escherichia coli	12	2 (16.7)	2 (16.7)	2 (16.7)	6 (50.0)	7 (58.3)	0.37 (0.05–0.82)
Enterococcus faecalis	17	3 (17.7)	3 (17.7)	7 (41.2)	4 (23.5)	10 (58.8)	0.27 (0.10–0.50)
Pseudomonas aeruginosa	26	0	0	0	26 (100.0)	26 (100.0)	0.43 (0.31–0.60)

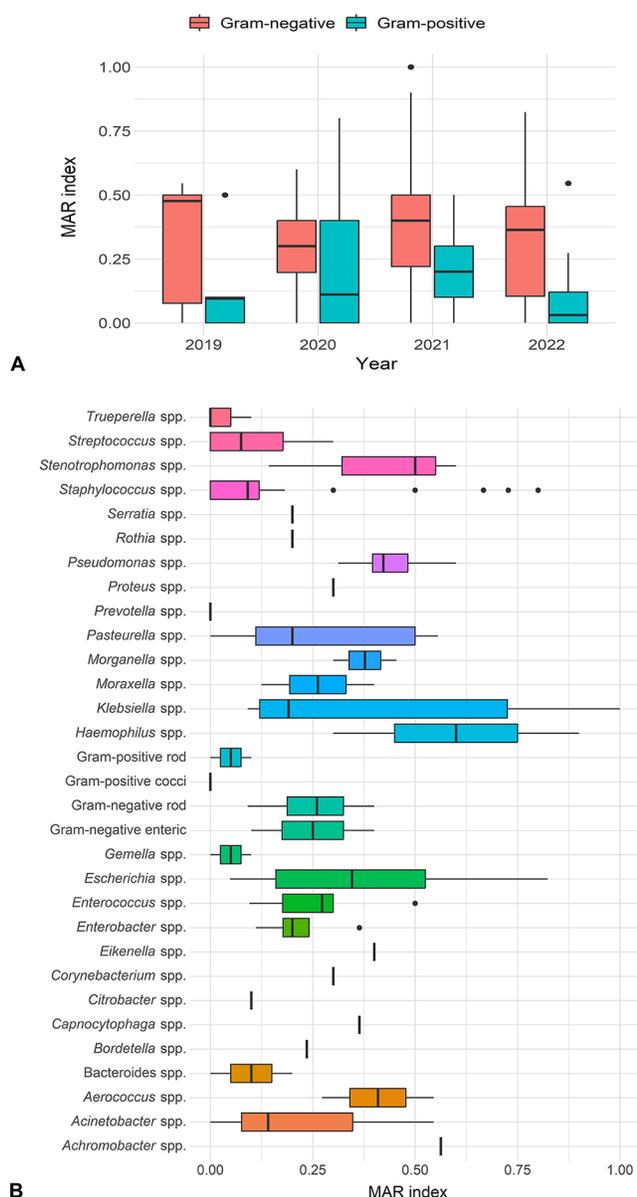MAR = multiple antibiotic resistance; MDR = multidrug resistance.

Figure 4. Multiple antimicrobial resistance (MAR) index for bacterial isolates recovered from 168 rabbit clinical samples in Hong Kong. **A.** MAR index of bacterial groups stratified by sampling year. **B.** MAR index of each bacterial species.

clinical samples in our study contrasts sharply with the higher percentage of gram-positive bacteria, such as *E. faecalis*, *S. intermedius*, and *S. aureus*. This difference may be attributed to the increased susceptibility of gram-negative bacteria to environmental conditions compared to their gram-positive counterparts.[31]

Our AST results revealed that 82.7% of bacterial isolates were resistant to ≥1 antimicrobial, with the highest resistance observed against penicillin (69.8%) and clindamycin (57.9%). Various levels of AMR have been reported for *E. coli*,[4,22] enterococci,[31] *P. multocida*,[13] and *S. aureus*[35] in pet rabbits. For example, a UK study isolated 1 *E. coli* from 13 pet rabbit

samples with resistance to tetracycline.[22] In contrast, a study in Portugal reported lower resistance levels among *E. coli* (11.4%) and enterococci (46.9%) isolates,[31] differing from our findings wherein a significant portion of *E. coli* isolates were resistant to multiple antimicrobials. The resistance level observed in our study, although lower, is comparable to that reported in wild (71.4% of *E. coli* isolates were resistant to 1 or 2 antimicrobials[8]) and food-producing animals.[30,33] Additionally, *E. coli* had one of the highest levels of MDR at 58.3%, aligning with data from domestic pets in Portugal (50%)[19] and sick companion animals in Singapore (57%).[16] The adaptability and effectiveness of the *E. coli* efflux system enables it to acquire and disseminate AMR determinants, contributing to resistance against multiple antimicrobial agents.[32]

Conversely, 66.7% of *S. aureus* isolates in our study had resistance to ≥1 antimicrobial, with none being methicillin-resistant *S. aureus* (MRSA) or having MDR. Although this level of resistance was higher than reported in European farmed rabbits,[3,35] the frequency of MDR isolates (92.3%) in those studies was greater, which can be explained by the more extensive use of antimicrobials in farmed rabbits.[3]

P. multocida is a significant pathogen often responsible for infections in rabbits, leading to conditions such as pneumonia, otitis media, orchitis, and pyometra.[9] We found that *P. multocida* was one of the most frequently isolated bacteria from clinical samples of rabbits, with 11% of these isolates displaying resistance to a single antimicrobial; 55.6% exhibited MDR. Our result is similar to a report from Brazil in which 47.8% of *P. multocida* isolates from rabbits had resistance to ≥1 antimicrobial.[13]

Our findings revealed high levels of MDR bacterial infections in clinical samples from rabbits in HK. A substantial proportion of these isolates had resistance to antimicrobial agents listed as highly important (such as clindamycin) for human medicine by the WHO.[7] Furthermore, most of the bacteria identified in our study can be transmitted from animals to their human caregivers. It is imperative to implement strategies aimed at mitigating these public health risks. This includes increasing awareness among pet owners about the prudent use of antimicrobials in treating rabbits, and promoting the utilization of antibiotic susceptibility profiles in veterinary clinics in HK.

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