# CHAPPER IV

## RESULTS

In this study, a questionnaire survey investigating questions on dairy cattle management, farm characteristics, and treatment strategies emphasizing the usage of antimicrobials was administrated among herd managers of 99 dairy farms located in five districts in Beijing (Figure 6), China in the period between November 2012 and April 2013. The questionnaires were distributed by local veterinarians during sampling or routine farm visit and questions were answered by the herd managers of farms or farm veterinarians. Moreover, fecal samples were collected in recruited farms and investigated concerning the occurrence of ESBLs.

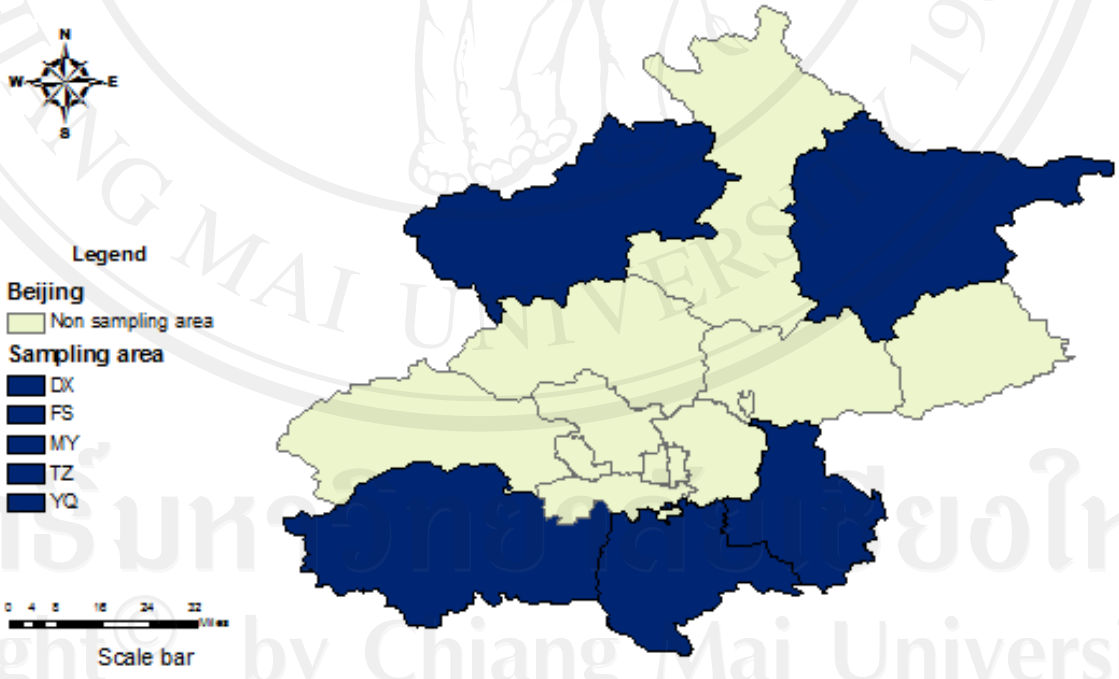


**Figure 6.** Sampling areas for studying ESBL-producing *E. coli* in dairy farms in Beijing.

DX=Daxing,FS=Fangshan, MY=Miyun, TZ=Tongzhou, YQ=Yanqing.

## 4.1 Questionnaire survey findings

### 4.1.1 General information of the dairy farms

The questionnaire survey provides an overview of the herd structure as well as milk yields. The dairy farms included in the study had a median size of 287 cows, ranging from 15 to 2736 animals. Table 7 gives an overview over the average number of calves, heifers, cows in the previous year and lactations per farm were 49, 56, 280 and 160, respectively. The median milk yield/cow and bulk milk SCC were 6100 kg and 315,000 cells/ml, respectively. The mean culling rate in the previous year was 14%, ranging from 3% to 74%.

**Table 7.** Descriptive statistics of farm data from 99 dairy farms in Beijing area.

| | N | Median | Mean | SD | IQR | Min | Max |
|---|---|---|---|---|---|---|---|
| No. of cows | 99 | 287 | 471.24 | 527.7 | 371.5 | 15.0 | 2736.0 |
| No. of calves | 95 | 49 | 85.44 | 101.6 | 100.0 | 2.0 | 615.0 |
| No. of heifers | 93 | 56 | 102.96 | 156.3 | 82.0 | 1.0 | 899.0 |
| No. of cows last year | 93 | 280 | 460.78 | 514.6 | 460.0 | 12.0 | 2621.0 |
| No. of lactation cows | 99 | 160 | 238.02 | 259.1 | 202 | 2.0 | 1310.0 |
| Milk yield/cow/year(kg) | 98 | 6100.0 | 6656.6 | 1849.9 | 2762.5 | 3721.0 | 11192.0 |
| SCC(x 1,000 cells per ml) | 48 | 300.0 | 315.4 | 113.9 | 150.0 | 100.0 | 600.0 |
| Average age of culled cow | 89 | 5.0 | 5.1 | 1.7 | 2.0 | 0.0 | 9.0 |
| Culling rate of last year | 81 | 0.14 | 0.16 | 0.12 | 0.10 | 0.03 | 0.74 |

Table 8 shows a profile of herd characteristics in the recruited farms summarized by herd size. In large farms, the average number of total cattle, calves, heifers, number of cattle in last year and number of milking cows are 1139, 200, 255, 1121, 548, respectively. In contrast, the numbers in small farms are 208, 42, 47, 205, and 116, respectively.

**Table 8.** Descriptive statistics of herd characteristics of 99 dairy farms in Beijng obtained by questionnaire survey. The shown data were summarized for different herd sizes. Large: great than 500 cattle, Small: equal and less than 500 cattle. Pop: population number.

| | Pop | | Pop_calve | | Pop_heifer | | Pop_last.year | | Pop_milking cows | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Large | Small | Large | Small | Large | Small | Large | Small | Large | Small |
| F. No. | 28 | 71 | 26 | 69 | 25 | 68 | 26 | 67 | 28 | 71 |
| Mean | 1139 | 208 | 200 | 42 | 255 | 47 | 1121 | 205 | 548 | 116 |
| Sd | 567.5 | 130.4 | 124.5 | 39.8 | 224.4 | 61.3 | 544.3 | 136.8 | 295.6 | 81.8 |
| Min | 508 | 15 | 131 | 2 | 56 | 1 | 520 | 12 | 200 | 2 |
| Median | 1040 | 214 | 168 | 30 | 176 | 29 | 1009 | 210 | 531 | 118 |
| Max | 2736 | 470 | 615 | 196 | 899 | 370 | 2621 | 600 | 1310 | 286 |

In large farms, the average yearly milk yield per cow was 8253.1 kg; the mean total year milk production was 5477.8 tons. The average yearly milk yield per cow and total year milk production in small farm were 6018 kg and 756.8 tons, respectively (Table 9 and Figure 7). Statistical analysis showed the 8253.1±1854.5 kg/year of average milk yield in large farms were significantly higher than the 6018±1420.3 kg/year of average milk yield in small farms ($p<0.001$). The somatic cell count in large farms were 318,000 cells/ml, while in small farms, the average somatic cell count were 313,000 cells/ml (Table 9, Figure 7). There was no significant difference between large and small farms ($p=0.8818$).

**Table 9.** Yearly milk yield per cow, total milk production and SCC in the respondent farms according to the questionnaire survey summarized by farm size.

| | Average yearly milk yield (kg) | | Total milk production (ton) | | SCC (x 1,000 cells per ml cells/ml) | |
|---|---|---|---|---|---|---|
| | Large | Small | Large | Small | Large | Small |
| F. No | 28 | 70 | 28 | 70 | 20 | 28 |
| Mean | 8253.1 | 6018 | 5477.8 | 756.8 | 318 | 313 |
| Sd | 1854.5 | 1420.3 | 3792.8 | 600.6 | 127 | 106 |
| Min | 5000 | 3721 | 1300 | 15 | 100 | 100 |
| Median | 8581 | 6000 | 5810 | 720 | 295 | 300 |
| Max | 11192 | 10700 | 15425 | 2500 | 600 | 500 |

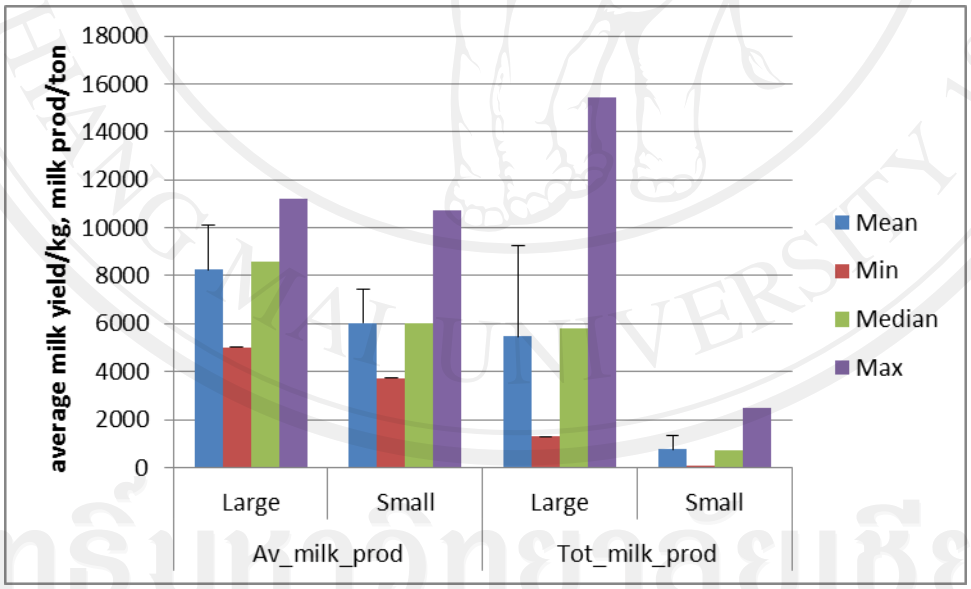


**Figure 7.** Yearly milk yield and total milk production in the respondent farms (n=98) according to the questionnaire survey summarized by farm size. Av_milk_prod: average year milk yield, Tot_milk_prod: total milk production.

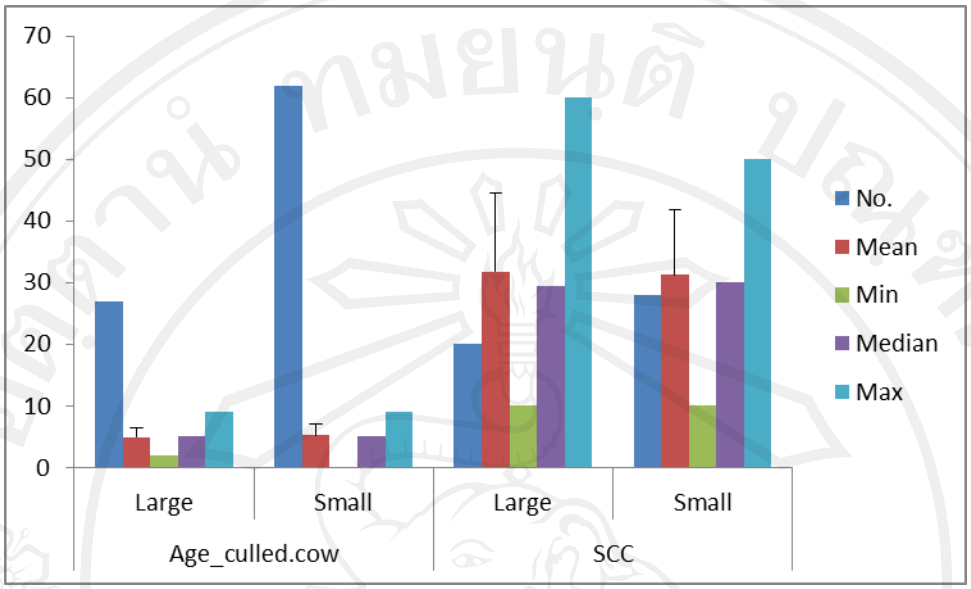


**Figure 8.** Age in years of culled cows and somatic cell count (SCC) (x 10,000 cells per ml cells/ml) investigated by questionnaire survey in the respondent farms in Beijing area summarized by farm size.

Table 10 and figure 8 showed the age of culled cows and culled rate in 2011 in the respondent farms in Beijing area summarized by farm size. The average age of culled cows in large farms were 4.9 years, while in small farms, the age of culled the cows were around 5.2 years. The average culling rate in large farms and small farms were 14% and 17%, respectively. The maximum culled rate in large farm was 25%, while in small farm was 74% in 2011.

**Table 10.** Age of culled cows and culling rate in the last year of the questionnaire survey carried out in respondent farms in Beijing area summarized by farm size.

| | Age of culled cow | | Culled rate in 2011 | |
|---|---|---|---|---|
| | Large | Small | Large | Small |
| F. No | 27 | 62 | 22 | 59 |
| Mean | 4.9 | 5.2 | 0.14 | 0.17 |
| Sd | 1.5 | 1.9 | 0.06 | 0.14 |
| Min | 2 | 0 | 0.04 | 0.03 |
| Median | 5 | 5 | 0.14 | 0.14 |
| Max | 9 | 9 | 0.25 | 0.74 |

The descriptive statistics of herd characteristics of respondent farms located in Beijing obtained by questionnaire survey summarized by ESBL producing *E. coli* test results were showed in table 11. The average population and milking cows population in ESBL producing *E. coli* negative farms was 416 and 217, while in positive farms was 756 and 342, respectively. The average yearly milk yield per cow and total milk production in ESBL negative farms were 6490 kg and 1795 tons, in contrast, in ESBL positive farms were 7506 kg and 3697.6 tons, respectively.

**Table 11.** Descriptive statistics of herd characteristics of respondent farms located in Beijing obtained by questionnaire survey summarized by ESBL producing *E. coli* positive and negative result. Av milk yield: average yearly milk yield per cow (kg), Pop_milking: population of milking cows. SCC: somatic cell count (x10000 cells/ml), Tot_milk _prod: total milk production in the farm(ton). Neg: ESBL producing *E. coli* negative, Pos: ESBL producing *E. coli* positive.

| | Population | | Av milk yield | | Pop_milking | | SCC | | Tot_milk_prod | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Neg | Pos | Neg | Pos | Neg | Pos | Neg | Pos | Neg | Pos |
| Farms | 83 | 16 | 82 | 16 | 83 | 16 | 37 | 11 | 82 | 16 |
| mean | 416 | 756 | 6490 | 7506 | 217 | 342 | 31.7 | 30.8 | 1795.0 | 3697.6 |
| sd | 423.3 | 858.3 | 1718.4 | 2294.2 | 228.0 | 374.5 | 11.8 | 10.5 | 2413.6 | 4751.9 |
| IQR | 357 | 1219.5 | 2400 | 3320 | 172 | 545.3 | 15 | 12.5 | 1405.3 | 6417.4 |
| Min | 15 | 15 | 3721 | 4575 | 2 | 10 | 10 | 10 | 15 | 50 |
| Median | 287 | 300 | 6025 | 7105 | 153 | 175 | 30 | 30 | 986 | 1500 |
| Max | 1930 | 2736 | 10920 | 11192 | 1310 | 1285 | 60 | 50 | 14296 | 15424.5 |
| P value | 0.1407 | | 0.1095 | | 0.215 | | 0.8036 | | 0.1371 | |

### 4.1.2 Common used antimicrobials

The most common used antimicrobials on the 75 respondent farms were penicillin which was administered on 48% of the respondent farms, while cephalosporin was administered on 36.0 %. The proportion of farms which had chosen erythromycin, gentamicin and penicillin, lincomycin varied from 1.3% to 4 %. The indications for choosing antimicrobials included mastitis, surgery, dystocia and metritis (Figure 9).

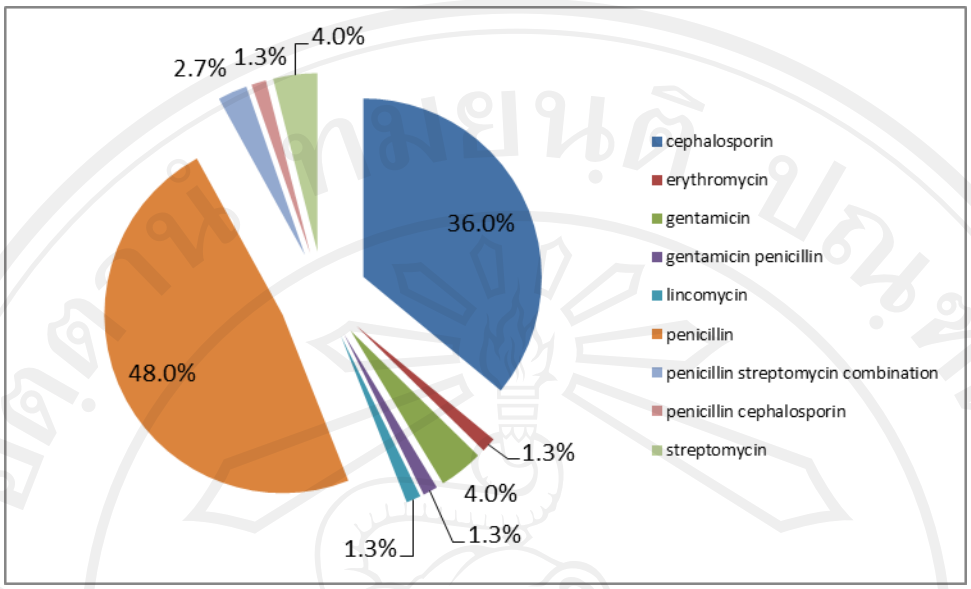


**Figure 9.** Commonly used antimicrobials in the response farms according to the questionnaire survey in 99 dairy farms in Beijing, China.

Concerning the farm size, there were 42.3 % of the respondent large farms frequently used cephalosporin and penicillin, respectively, while 15.4 % farms selected others antimicrobial agents such as gentamicin and streptomycin. However, 32.7% of the respondent small farms commonly used cephalosporin in their routine disease control and treatment procedure; 51.0% small farms selected penicillin and 16.3% selected others antimicrobial agents (Table 12).

**Table 12.** The most common used antimicrobials in the respondent farms in Beijing area summarized by farm size.

| | Cephalosporin | | Penicillin | | Others | | Total Farms | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| Large farms | 11 | 42.3% | 11 | 42.3% | 4 | 15.4% | 26 | 100.0% |
| Small farms | 16 | 32.7% | 25 | 51.0% | 8 | 16.3% | 49 | 100.0% |

### 4.1.3 Animal health status and frequencies of disorders in dairy farms

Questions were asked to farm managers or farm veterinarians to estimate the occurrence of certain disorders among their herd by choosing one of the three criteria: never, sometimes and frequent. The list on the form included lameness, metritis, dystocia, surgery, cow diarrhea and calves diarrhea occurring in dairy cows and calves that likely require the use of antimicrobials (Table 13 and 14). In the respondent farms, in 26.8% of farms lameness never occurred, in 71.1% sometimes and in 2.1% it happened frequently. For metritis, percentage of the occurrence frequencies of never, sometimes and frequent were 11.8%, 82.8% and 5.4%, respectively, while for dystocia, occurrence frequencies for the three criteria were 28.6%, 71.4% and 0%, respectively; for surgery intervention, were 57.3%, 41.7% and 1.0%, respectively; for cow diarrhea, were 27.6%, 71.4% and 1.0%, respectively; as far as occurrence of calves diarrhea, the percentage were 15.5%, 84.5% and 0.0%, respectively.

**Table 13.** Farm veterinarians and herd managers'estimations of the occurrence of lameness, metritis and dystocia in their farm. Results of a questionnaire among herd manager and farm veterinarians from 99 dairy farms in Beijing, China. Respondents could choose one of the following criteria: never, sometimes and frequent.

| | Lameness | | Metritis | | Dystocia | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| Never | 26 | 26.8% | 11 | 11.8% | 28 | 28.6% |
| Sometimes | 69 | 71.1% | 77 | 82.8% | 70 | 71.4% |
| Frequent | 2 | 2.1% | 5 | 5.4% | | 0.0% |
| Respondents | 97 | 100.0% | 93 | 100.0% | 98 | 100.0% |

**Table 14.** Farm veterinarians and herd managers'estimations of the occurrence of surgery interventions, cow diarrhea and calves diarrhea in their farm. Results of a questionnaire among herd manager and farm veterinarians from 99 dairy farms in Beijing, China. Respondents could choose one of the following criteria: never, sometimes and frequent.

| | Surgery | | Cow diarrhea | | Calves diarrhea | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| Never | 55 | 57.3% | 27 | 27.6% | 15 | 15.5% |
| Sometimes | 40 | 41.7% | 70 | 71.4% | 82 | 84.5% |
| Frequent | 1 | 1.0% | 1 | 1.0% | 0 | 0.0% |
| Respondents | 96 | 100.0% | 98 | 100.0% | 97 | 100.0% |

### 4.1.4 Antimicrobials usage in respondent farms

The information on antimicrobials administrated for the treatment of common diseases including lameness, metritis, dystocia, surgery intervention, cow diarrhea and calves diarrhea are shown in table 15 and table 16. 54.7% (52/95) of the farms administrated antimicrobials to treat lameness, metritis 77.8% (77/99), and dystocia 37.4% (37/99), surgery intervention 45.4% (44/97), cow diarrhea 54.1% (53/98), and calves diarrhea 71.1% (69/97). For the farms never used antimicrobials for the treatment of diseases, there were varies in different diseases. The lowest percentage of farms never used antimicrobials was metritis and the highest was dystocia that the respondent farms never used antimicrobials.

**Table 15.** Frequencies of antimicrobials used for treating lameness, metritis and dystocia in farms summarized from a questionnaire among herd managers from 99 dairy farms in Beijing, China. Respondents could choose between one of the following criteria: never, sometimes and frequent.

| | AB used for lameness | | AB used for metritis | | AB used for dystocia | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| Never | 43 | 45.3% | 22 | 22.2% | 62 | 62.6% |
| Sometimes | 51 | 53.7% | 72 | 72.7% | 35 | 35.4% |
| Frequent | 1 | 1.1% | 5 | 5.1% | 2 | 2.0% |
| Respondents | 95 | 100.0% | 99 | 100.0% | 99 | 100.0% |

**Table 16.** Frequencies of antimicrobials used for treating surgery, cow diarrhea and calve diarrhea in farms summarized from a questionnaire among herd managers from 99 dairy farms in Beijing, China. Respondents could choose between one of the following criteria: never, sometimes and frequent.

| | AB used for surgery | | AB used for cow diarrhea | | AB used calve diarrhea | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| Never | 53 | 54.6% | 45 | 45.9% | 28 | 28.9% |
| Sometimes | 28 | 28.9% | 51 | 52.0% | 65 | 67.0% |
| Frequent | 16 | 16.5% | 2 | 2.0% | 4 | 4.1% |
| Respondents | 97 | 100.0% | 98 | 100.0% | 97 | 100.0% |

### 4.1.5 Antimicrobials category and proportion used in different disorders

The farms which administrated antimicrobials for the treatment of disorders were asked to give the exact antimicrobial name. 40.7% of respondent farms administrated cephalosporin and 33.3% farms gave penicillin during treatment of lameness (Table

17). For treatment of cow diarrhea, 51.9% of the respondent farms (Table 18) choose gentamicin, while 63.9% of respondent farms selected gentamicin to treat calves diarrhea (Table 19). 40.9% of the respondent farms answered the most frequently used antimicrobial for the treatment of dystocia was penicillin (Table 20). However, during surgery, 32.0% of farms preferred to cephalosporin and 40.0% farms indicated penicillin (Table 21).

**Table 17.** Antimicrobials and proportion used for the treatment of lameness in 99 dairy farms in Beijing, China.

| Antimicrobials used for lameness | Farms | % |
|---|---|---|
| Cephalosporin | 11 | 40.7% |
| Gentamicin | 1 | 3.7% |
| Gentamicin, Penicillin | 1 | 3.7% |
| Penicillin | 9 | 33.3% |
| Streptomycin, Penicillin, Cephalosporin | 3 | 11.1% |
| Terramycin | 2 | 7.4% |
| Respondents | 27 | 100.0% |

**Table 18.** Antimicrobials and proportion used for the treatment of cow diarrhea in 99 dairy farms in Beijing, China.

| Antimicrobials used for cow diarrhea | Farms | % |
|---|---|---|
| Ampicillin | 1 | 3.7% |
| Gentamicin | 14 | 51.9% |
| Gentamicin, Norfloxacin | 1 | 3.7% |
| Gentamicin, Penicillin | 2 | 7.4% |
| Gentamicin, Sulfanamide | 1 | 3.7% |
| Mequindox | 2 | 7.4% |
| Norfloxacin | 1 | 3.7% |
| Streptomycin | 1 | 3.7% |
| Sulfadiazine | 3 | 11.1% |
| Tetracycline | 1 | 3.7% |
| Respondents | 27 | 100.0% |

**Table 19.** Antimicrobials and proportion used for the treatment of calves' diarrhea in 99 dairy farms in Beijing, China.

| Antimicrobials used for calves diarrhea | Farms | % |
|---|---|---|
| Ampicillin | 1 | 2.8% |
| Gentamicin | 23 | 63.9% |
| Gentamicin, Fluorifnecicol | 1 | 2.8% |
| Gentamincin, Norfloxacin | 1 | 2.8% |
| Gentamincin, Cephalosporin, Sulfanamide | 2 | 5.6% |
| Long Acting Terramycin | 1 | 2.8% |
| Mequindox | 2 | 5.6% |
| Mequindox, Gentamicin | 1 | 2.8% |
| Gentamicin, Norfloxacin | 3 | 8.3% |
| Penicillin, Gentamicin, Sulfanamide | 1 | 2.8% |
| Respondents | 36 | 100.0% |

**Table 20.** Antimicrobials and proportion used for the treatment of dystocia in 99 dairy farms in Beijing, China.

| Antimicrobials used for dystocia | Farms | % |
|---|---|---|
| Ampicillin | 1 | 4.5% |
| Cephalosporin | 4 | 18.2% |
| Lincomycin | 1 | 4.5% |
| Penicillin | 9 | 40.9% |
| Penicillin, Streptomycin | 3 | 13.6% |
| Penicillin, Cephalosporin | 1 | 4.5% |
| Terramycin | 2 | 9.1% |
| Terramycin, Cephalosporin | 1 | 4.5% |
| Respondents | 22 | 100.0% |

**Table 21.** Antimicrobials and proportion used during surgery intervention 99 dairy farms in Beijing, China.

| Antimicrobials used for surgery | Farms | % |
|---|---|---|
| Ampicillin | 1 | 4.0% |
| Cephalosporin | 8 | 32.0% |
| Gentamicin | 1 | 4.0% |
| Penicillin | 10 | 40.0% |
| Penicillin, Streptomycin | 3 | 12.0% |
| Peincillin, Cephalosporin | 2 | 8.0% |
| Respondents | 25 | 100.0% |

**4.1.6 Criteria for administrating antimicrobials for treatment of mastitis**

The decision to treat cows affected with mastitis by administration of antimicrobials depends either on the presence of clinical symptoms or visible alterations of the milk. 44.3% of respondent farms administrated antimicrobials once there were visible alterations present in the milk and 27.8% of the farms choose antimicrobial treatment for mastitis when the general conditions were affected. There were 10.1% of farms used antimicrobial treatments for mastitis when the SCC tested result higher than the standard (Table 22).

**Table 22.** Criteria for using antimicrobials for the treatment of mastitis summarized according to the questionnaire survey in 99 dairy farms in Beijing, China.

| Criteria for using AB for mastitis | Farms | % |
|---|---|---|
| High SCC in single cows | 8 | 10.1% |
| Visible alterations of the milk only | 35 | 44.3% |
| General condition was affected | 22 | 27.8% |
| Others | 14 | 17.8% |
| Respondents | 79 | 100.0% |

### 4.1.7 Duration time for using antimicrobial for treatment of disorders

The duration time that farms choose antimicrobials for treatment of disorder were various from farm to farm. The majority of the farms (64.3%) have used antimicrobials for 3-4 days, 20.4% farms for more than 4 days (Table 23).

**Table 23.** Duration time for using antimicrobials for the treatment of disorders obtained from questionnaire survey in 99 dairy farms in Beijing, China.

| Duration time for using antimicrobial | Farms | % Farms |
|---|---|---|
| 1-2 Days | 10 | 10.2% |
| 3-4 Days | 63 | 64.3% |
| More than 4 days | 20 | 20.4% |
| Others | 5 | 5.1% |
| Respondents | 98 | 100.0% |

### 4.1.8 Indication of using antimicrobial for disorders in dairy farms

According to the questionnaire survey, there were 32.8% respondent farms choose mastitis as the indication to use antimicrobials, 31.0% farm used antimicrobials once found the cattle inflammation , 19.0% of the farms selected antimicrobials treatment when metritis occurrence(Table 24).

**Table 24.** Indication of using antimicrobial for disorders obtained from questionnaire survey in 99 dairy farms in Beijing, China.

| Indication of using antimicrobial | Farms | % Farms |
|---|---|---|
| Mastitis | 19 | 32.8% |
| Inflammation | 18 | 31.0% |
| Metritis | 11 | 19.0% |
| Surgery | 4 | 6.9% |
| Others | 6 | 10.3% |
| Respondents | 58 | 100.0% |

### 4.1.9 Management of cattle treated with antimicrobials

From the 87 respondent farms, 63.2% of farms separated the cattle treated with antimicrobials from those without application of antimicrobials. 24.1% of the farms did not separate the cattle when they administrated antimicrobials (Table 25).

**Table 25.** Manage cattle when using antimicrobials for treatment of disorders. Results summarized from questionnaire survey in 99 dairy farms in Beijing, China.

| Management of cattle when using antimicrobial | Farms | % Farms |
|---|---|---|
| Separated from the group | 55 | 63.2% |
| Remain in their group | 21 | 24.1% |
| Both options were applied | 10 | 11.5% |
| Others method | 1 | 1.1% |
| Respondents | 87 | 100.0% |

**4.2 Laboratory test results**

**4.2.1 Bacterial isolation and confirmation**

ESBL-producing *E. coli* was isolated in 16 of 99 sampled farms by the direct plate method resulting in a herd prevalence of ESBL-producing *E. coli* of 16.2% for the investigated region. All these isolates were confirmed as *E. coli* by using API 20 E kit (99.8%). Out of 28 large farms in 6 farms ESBL-producing *E. coli* could be found resulting in a prevalence of 21.4% (6/28). 10 out of 61 small farms were test positive for ESBL-producing *E. coli* with a prevalence of 14.1% (10/61). No significant difference was found between the prevalence in large and small farms (P value 0.371).

**4.2.2 Screening and confirmatory tests for ESBLs**

Confirmatory tests for ESBLs were carried out by using disk diffusion method and the results show that all 16 strains could be confirmed as ESBL-producers (Table 26).

**Table 26.** Zone diameter and zone diameter increase results for either antimicrobial agent tested in combination with clavulanic acid versus the zone when tested alone for cefotaxime or ceftazidime.

| AB agents | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 | F13 | F14 | F15 | F16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CTX | 13 | 13 | 0 | 14 | 0 | 11 | 0 | 14 | 8 | 11 | 8 | 0 | 13 | 8 | 11 | 11 |
| CTX/CA | 31 | 31 | 31 | 29 | 33 | 31 | 31 | 29 | 31 | 30 | 29 | 31 | 30 | 32 | 30 | 29 |
| zoneincr | 18 | 18 | 31 | 15 | 33 | 20 | 31 | 15 | 23 | 19 | 21 | 31 | 17 | 24 | 19 | 18 |
| CAZ | 25 | 19 | 14 | 20 | 21 | 13 | 14 | 20 | 13 | 18 | 12 | 9 | 15 | 16 | 15 | 14 |
| CAZ/CA | 31 | 31 | 34 | 26 | 30 | 31 | 34 | 26 | 25 | 27 | 28 | 29 | 28 | 28 | 30 | 29 |
| zoneincr | 6 | 12 | 20 | 6 | 9 | 18 | 20 | 6 | 12 | 9 | 16 | 20 | 13 | 12 | 15 | 15 |

CTX-cefotaxime, CTX/CA-cefotaxime+clavulanic acid, CAZ-ceftazidime,CAZ/CA-ceftazidime+clavulanic acid, zoneincr-zone diameter increase (mm)

### 4.2.3 Antimicrobial susceptibility testing

In the present study, the 16 ESBL-producing *E. coli* strains from fecal samples were tested against 14 standard antimicrobial agents. Table 27 shows the results of antimicrobial susceptibility tests.

**Table 27.** Susceptibility of 16 ESBL-producing *E. coli* strains isolated from fecal samples originating from 99 farms to 14 standard antimicrobials agents. Figures in brackets indicate inhibition zone diameters in intermediate isolates.

| Sample ID | S | C | CN | CTX | CAZ | W | TE | SMZ | CIP | K | NA | AMP | SXT | AMC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S01 | - | - | R | R | - | - | - | - | - | I(17) | - | R | - | - |
| S02 | - | - | - | R | - | - | - | - | - | - | - | R | - | - |
| S03 | I(12) | - | I(13) | R | R | - | I(14) | R | R | R | - | R | R | - |
| S04 | R | R | R | R | - | R | R | R | R | R | R | R | R | - |
| S05 | R | R | R | R | - | R | R | R | - | R | - | R | R | - |
| S06 | R | R | R | R | R | R | R | R | I(17) | R | R | R | R | - |
| S07 | I(12) | - | I(13) | R | R | S | I(14) | R | R | R | - | R | R | - |
| S08 | R | R | R | R | R | R | R | R | R | R | R | R | R | - |
| S09 | R | R | I(14) | R | R | R | R | R | - | R | - | R | R | - |
| S10 | R | - | R | R | - | R | - | R | R | R | - | R | R | - |
| S11 | R | - | R | R | R | R | - | R | R | R | - | R | R | - |
| S12 | R | - | R | R | R | - | I(14) | R | R | R | - | R | S | - |
| S13 | R | - | R | R | I(15) | R | R | R | R | R | R | R | R | I(15) |
| S14 | R | - | - | R | I(16) | - | - | - | R | I(16) | - | R | - | - |
| S15 | R | - | I(13) | R | I(15) | - | - | - | - | R | - | R | - | - |
| S16 | R | R | I(13) | R | R | - | R | - | - | R | R | R | - | - |

R- resistant, -- susceptible, I-intermediate. S-Streptomycin, C-Chloramphenicol, CN-Gentamicin, CTX-Cefotaxime, CAZ-Ceftazidime, W-Trimethoprim, TE-Tetracycline, SMZ-Sulfamethoxazole, CIP-Ciprofloxacin, K-Kanamycin, NA- Nalidixic acid, AMP-Ampicillin, SXT-Sulfamethoxazole +Trimethoprim, AMC-amoxicillin + Clavulanic acid.

A summarized overview about the resistance situation of the 16 ESBL-producing *E. coli* against the different investigated antimicrobials gives Figure 10.

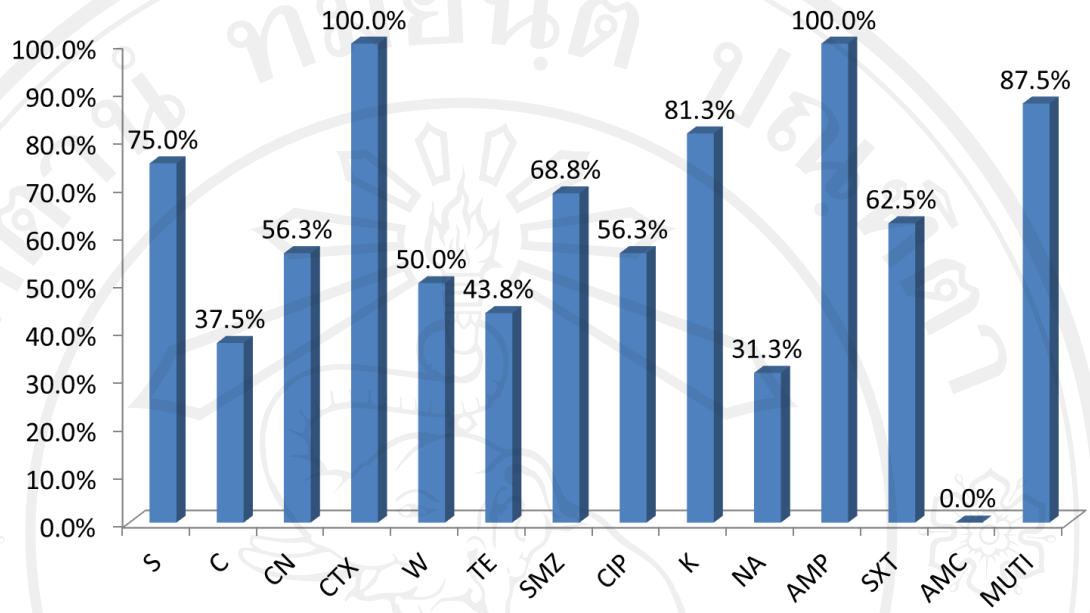


**Figure 10.** Proportions of susceptibility to 13 antimicrobial agents as determined by disk diffusion of 16 ESBL-producing *E. coli* strains isolated from feces obtained at sampling on a single occasion from 99 dairy farms in Beijing, China. S-Streptomycin, C-Chloramphenicol, CN-Gentamicin, CTX-Cefotaxime, W-Trimethoprim, TE-Tetracycline, SMZ-Sulfamethoxazole, CIP-Ciprofloxacin, K-Kanamycin, NA-Nalidixic acid, AMP-Ampicillin, SXT-Sulfamethoxazole +Trimethoprim, AMC-amoxicillin + Clavulanic acid.

### 4.3 Risk factors analysis

#### 4.3.1 Farm administration

Risk factors related with the farm administration measures were analyzed using R studio software. Table 28 shows the associations of positive results concerning ESBL-producing *E. coli* and risk factors including farm scale, feeding waste milk to calves, raising other animals together with cattle, feeding antimicrobial residual milk to calves and document recording when using antimicrobials. For the risk factor

feeding of milk with antimicrobial residuals to calves, an odds ratio of 4.74 ( 95% CI: 1.35-19.25) indicates that farms that feed antimicrobial residual milk to calves had a 4.74 times higher risk of detecting ESBL-producing *E. coli* compared to farms that did not feed antimicrobial residual milk to calves. For the other assumed risk factors, we did not find any association with the detection of ESBLs-producing *E. coli* in dairy farms (Table 28).

**Table 28.** Associations between detection of ESBL-producing *E. coli* in fecal samples and some administration measures in 99 dairy farms in Beijing, China.

| Risk factors (Farm administrations) | | No. farms | Prevalence % | OR | 95%CI of OR |
|---|---|---|---|---|---|
| Farm level | Large | 28 | 21.4 | 1.65 | 0.44-5.75 |
| | Small | 71 | 14.1 | | |
| Feed Waste milk | Yes | 33 | 15.2 | 0.89 | 0.22-3.14 |
| | No | 66 | 16.7 | | |
| Other animals raised | Yes | 25 | 16 | 0.98 | 0.21-3.73 |
| | No | 74 | 16.2 | | |
| Feed antimicrobial residual milk to calves | Yes | 37 | 29.73 | 4.74 | 1.35-19.25 |
| | No | 62 | 8.06 | | |
| Record for antimicrobial usage | Yes | 73 | 15.1 | 0.75 | 0.21-3.07 |
| | No | 26 | 19.2 | | |

### 4.3.2 Antimicrobials using status

In this study, some risk factors related to antimicrobials usage, including rotation using antimicrobials, using slow release antimicrobials, frequency of using cephalosporin, antimicrobials used in some disorders and use measure for treatment of mastitis were analyzed. The OR of frequently using cephalosporin is 3.85 (95% CI: 1.01-14.66), indicated that farms which frequently used antimicrobials had a 3.85

times higher risk of detecting ESBL-producing *E. coli* compared to farms that did not frequently used this antibiotic substance. Similarly, the OR of the risk factor of treating four teats instead of only the diseased teat is 5.01 (95% CI: 1.43-20.41), means that farms treat all the four teats including healthy teats when the cow got mastitis had 5.01 time higher risk of detecting ESBLs-producing *E. coli* (Table 29).

**Table 29.** Associations between the detection of ESBL-producing *E. coli* in fecal samples and antimicrobials uing status in 99 dairy farms in Beijing, China.

| Risk factors (Antimicrobial using status) | | No. farms | Prevalence % | OR | 95%CI of OR |
|---|---|---|---|---|---|
| Rotating use of antimicrobials | Yes | 64 | 12.5 | 0.49 | 0.14-1.66 |
| | No | 35 | 22.9 | | |
| Contineous use of antimicrobial until cured | Yes | 90 | 16.7 | 1.59 | 0.19-75.64 |
| | No | 9 | 11.1 | | |
| Using slow release antimicrobials | Yes | 24 | 25 | 2.15 | 0.56-7.63 |
| | No | 75 | 13.3 | | |
| Using antimicrobials for the treatment of metritis | Yes | 77 | 16.9 | 1.28 | 0.31-7.74 |
| | No | 22 | 13.6 | | |
| Using antimicrobials for the treatment of dystocia | Yes | 37 | 24.3 | 2.5 | 0.74-8.82 |
| | No | 62 | 11.3 | | |
| Using antimicrobials for cow diarrhea | Yes | 53 | 17 | 1.11 | 0.33-3.87 |
| | No | 45 | 15.6 | | |
| Using antimicrobials during surgery intervention | Yes | 44 | 20.5 | 1.68 | 0.5-5.88 |
| | No | 53 | 13.2 | | |
| Using antimicrobials to treat lameness | Yes | 52 | 17.3 | 1.08 | 0.32-3.77 |
| | No | 43 | 16.3 | | |
| Using antimicrobials to treat calves diarrhea | Yes | 68 | 19.1 | 1.96 | 0.48-11.65 |
| | No | 28 | 10.7 | | |
| Frequently use cephalosporin | Yes | 27 | 25.9 | 3.85 | 1.01-14.66 |
| | No | 48 | 8.3 | | |
| Treat four teats instead of diseased teats for mastitis | Four teats | 36 | 30.6 | 5.01 | 1.43-20.41 |
| | Diseased | 63 | 7.9 | | |
| Prevent disease using antimicrobials for dried cow | Yes | 40 | 22.5 | 2.14 | 0.64-7.51 |
| | No | 59 | 11.9 | | |