

# Prevalence and antibiotic resistance of coagulase-negative *Staphylococci* isolated from poultry farms in three regions of Ghana

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**Abstract:** The use of antibiotics in animal production has been associated with the development and spread of antibiotic-resistant organisms including commensals. Coagulase-negative *Staphylococcus* (CoNS) species, which were until recently considered non-pathogenic, have been associated with opportunistic infections and high resistance to several antibiotics. This study sought to determine the prevalence, identity, and phenotypic resistance of coagulase-negative *Staphylococcus* spp. isolated from some selected poultry farms and farm workers in the Ashanti, Brong Ahafo, and Greater Accra regions of Ghana. Poultry litter samples and oral swabs of poultry farm workers were collected, from which bacterial species were isolated, identified, and analyzed. Various selective media were used for the presumptive identification of the different species. Confirmation of bacterial identity was done using matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry. Antibiotic susceptibility testing of the isolates was performed using the Kirby-Bauer disk diffusion method. Zones of growth inhibition were interpreted based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Two hundred and fifty-six coagulase-negative *Staphylococcus* spp., comprising *S. sciuri* (42.97%), *S. lentus* (35.94%), *S. gallinarum* (6.64%), *S. xylosum* (4.30%), *S. haemolyticus* (3.91%), *S. saprophyticus* (1.95%), and *S. cohnii* (0.39%) were confirmed by MALDI-TOF. CoNS were isolated from samples from the Brong Ahafo (48.83%), Ashanti (33.59%), and Greater Accra (17.78%) regions. Isolates from poultry litter constituted 55.47%, and farm workers 44.53%. All the isolates were susceptible to amoxicillin/clavulanic acid and amikacin. The isolates exhibited high resistance toward tetracycline (57.03%), doxycycline (43.75%), and oxacillin (43.36%). Multi-drug resistance (MDR) was observed in 19.14% of the isolates. MDR was higher in isolates obtained from poultry farm workers (61.22%) than isolates from poultry litter (38.78%). The above findings call for stricter monitoring of antibiotic usage in both animal production and in humans.

**Keywords:** poultry farms, antibiotic resistance, antibiotics, antimicrobials

## Introduction

The bacteria genus *Staphylococcus* is a Gram-positive, facultative anaerobe, which is round in shape and appears in clusters.<sup>1</sup> *Staphylococcus* spp. are also known to be non-motile, non-spore, glucose fermenting, and catalase producing.<sup>2</sup> *Staphylococcus aureus* is the most commonly known member of the family, and the most commonly known coagulase-positive strain.<sup>1</sup> Members of the *Staphylococcus* genus that do not produce coagulase are known as coagulase-negative *Staphylococci* (CoNS). Until recently, CoNS were considered as the non-pathogenic members of the genus and thus were not of much interest to the research community. However, due to their implication

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in infections in both humans and animals, research interest in CoNS has increased over the past decade.<sup>3-5</sup> In addition, CoNS have, over the last decade, developed resistance to multiple antibiotics,<sup>4,6,7</sup> making their study worthwhile, especially, since they are known commensals and could be prevalent in most environments.<sup>5,8</sup> The economic burden of Staphylococcal infections in animal husbandry includes decreased weight gain, drop in egg production (with respect to poultry), mortality, condemnation at slaughter, and lameness, among others.<sup>9,10</sup>

In Ghana, a developing country, with a growing population of about 25 million people and a land area of 385,500 square kilometers, agriculture provides employment and serves as the major source of livelihood for over 55% of the population.<sup>11</sup> Several individuals engage in livestock production as a means of protein (meat and eggs) supplementation in the home, and also as a source of income.<sup>12,13</sup> Commercial poultry production in Ghana is highly concentrated in the Ashanti, Brong Ahafo, and Greater Accra regions and these three regions contribute to almost 70% of commercial poultry production in the country.<sup>14,15</sup>

However, commercial poultry production in Ghana is challenged with occurrences of bacterial, viral, and parasitic infections.<sup>16</sup> This compels poultry farmers to employ several antimicrobial agents, including essential antibiotics, on their farms.<sup>13,16</sup> Studies have also shown that the farmers in the said regions employ these antibiotics even in the management of viral infections.<sup>13</sup> The misuse of antibiotics in animal production has been closely associated with the development and spread of antibiotic-resistant organisms, including non-pathogenic species and commensals.<sup>8,17</sup>

Notwithstanding the fact that research interest in CoNS has increased in recent years, there is very little data on their prevalence and resistance profiles in Ghana. Information on CoNS is currently unavailable, especially in areas where misuse of antibiotics could lead to selection pressure, development of resistance, and spread of resistant strains. Hence, the aim of this study was to determine the prevalence and antibiograms of coagulase-negative *Staphylococcus* spp. isolated from poultry bedding materials (poultry litter) and poultry farm workers in the Ashanti, Brong Ahafo, and Greater Accra regions of Ghana.

## Materials and methods

### Ethical clearance and approval

Ethical clearance for the study was obtained from the Committee on Human Research Publications and Ethics (CHRPE), Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. In addition, written consent was obtained from all participants.

### Selection of farms

A total of 400 poultry farms were randomly selected from the three regions as previously described by Boamah et al.<sup>13</sup> Sixty-one percent of the farms were located in the Ashanti region, 28.5% in the Brong Ahafo region, and 10.5% in the Greater Accra region. Eighty-two (20.5%) of the farms were small scale, 254 (63.5%) medium, and 64 (16%) large, as categorized by the Food and Agriculture Organisation (FAO).<sup>12</sup>

### Sampling from poultry bedding and poultry workers

One gram of poultry bedding material (litter) was aseptically collected from three different points in a pen, in sterile sample containers. In a situation where a farm had less than five pens, samples were collected from at least three different pens, and from farms with more than five pens, samples were collected from at least five different pens. Oral swabs from farm workers who had worked on the farm for at least 1 month, and those directly involved in the day-to-day management of birds in the farm were also taken. The samples were appropriately labeled and transported to the laboratory in a cold box.

### Isolation of CoNS species

Five and ten milliliters of trypticase soy broth (TSB) (Thermo Fisher Scientific, Waltham, MA, USA) were added to the samples from humans and poultry litter respectively, and then incubated at 37°C for 24 h. CoNS were isolated from the media by methods described by Reynolds<sup>18</sup> and Kateete et al.,<sup>19</sup> with slight modifications. Five hundred microliters suspension of TSB was aseptically transferred into 10 mL Mueller-Hinton broth (Thermo Fisher Scientific), supplemented with 6.5% NaCl, and incubated at 37°C for 24 h. After incubation, 10 µL was spread on 20 mL mannitol salt agar (MSA) (Thermo Fisher Scientific), and incubated at 37°C for 24 h. Well-isolated colonies on MSA medium were aseptically streaked onto 20 mL plates of *Staphylococcus-aureus*-select (SaSelect), (Bio-Rad Laboratories Inc., Hercules, CA, USA) and incubated at 37°C for 24 h. Bluish, purple-like, and whitish colonies grown on SaSelect were further streaked on 20 mL nutrient agar (Thermo Fisher Scientific) enriched with 5% sheep blood and then incubated at 37°C for 24 h. Identity of non-hemolytic colonies was then confirmed by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry.

### Confirmation of isolates by MALDI-TOF mass spectrometry

Twenty-four hour cultures of non-hemolytic colonies on blood agar plates were used. Isolated colonies were uni-

formly spread on the MALDI-TOF slide to form a thin film and then covered with 1  $\mu$ L of ready-to-use  $\alpha$ -cyano-4-hydroxycinnamic acid and allowed to air-dry. *Escherichia coli* 25922 was used as the control strain. The MALDI-TOF slide was loaded into the VITEK MS (BioMérieux Corporate, Paris, France) for identification. The identity of the isolate was determined using Saramis® program (BioMérieux Corporate). Isolates with identities >99% were confirmed as CoNS. *S. aureus* 25923, *E. coli* ATCC 25922 (American Type Culture Collection, Manassas, VA, USA), and all other strains of CoNS were stored at  $-80^{\circ}\text{C}$  until needed.

## Antibiotic susceptibility testing

Antibiotic sensitivity of the *Staphylococcus* spp. was determined using the disk diffusion method described by EUCAST<sup>20</sup> and Bauer et al.<sup>21</sup> Twenty-four hour colonies of *Staphylococci* growing on blood agar plates were used for the susceptibility testing. To ensure a good representation of the isolates in the culture, approximately five to seven well-separated colonies were picked and suspended in 5 mL sterile distilled water and vortexed at high speed until the suspension was uniform. The turbidity of the suspension was determined using a nephelometer already calibrated to 0.5 McFarland. The turbidity of the suspensions were then adjusted appropriately to 0.5 McFarland with the addition of more colonies or sterile distilled water.

A sterile cotton swab was soaked in the inoculum and rotated twice against the inner side of the test tube to remove excess liquid. The swab was used to streak the entire surface of 20 mL MHA plate whilst rotating the plate at an angle of  $60^{\circ}$  with repeated streaking (three times in total). With the aid of a disk dispenser, antibiotic disks (benzyl penicillin

1 unit; ampicillin 10  $\mu$ g; oxacillin 1  $\mu$ g; cefoxitin 30  $\mu$ g; trimethoprim/sulfamethoxazole 25  $\mu$ g; ciprofloxacin 5  $\mu$ g; norfloxacin 10  $\mu$ g; gentamicin 10  $\mu$ g; erythromycin 15  $\mu$ g; tetracycline 30  $\mu$ g; doxycycline 30  $\mu$ g; chloramphenicol 30  $\mu$ g) purchased from Thermo Fisher Scientific, were placed on the surface of the MHA. The plates were incubated at  $37^{\circ}\text{C}$  for 20 h. Zones of growth inhibition (mm) were measured and recorded. Susceptibility of the isolates to different antibiotics was determined and classified according to EUCAST.<sup>20</sup>

## Results

### Distribution of different *Staphylococcus* spp. in the selected farms

Of the 368 presumptive *Staphylococcal* colonies isolated using selective media, 256 (69.57%) were confirmed by MALDI-TOF mass spectrometry as coagulase-negative *Staphylococcus* spp. One hundred and fourteen (44.53%) were from farm workers whilst isolates from the poultry bedding material constituted 55.47%. Seven different CoNS species were identified and these include *S. sciuri* (42.97%), *S. lentus* (35.94%), *S. gallinarum* (6.64%), *S. xylosus* (4.30%), *S. haemolyticus* (3.91%), *S. saprophyticus* (1.95%), and *S. cohnii* (0.39%) (Table 1).

### Antibiotic sensitivity profiles of isolated CoNS

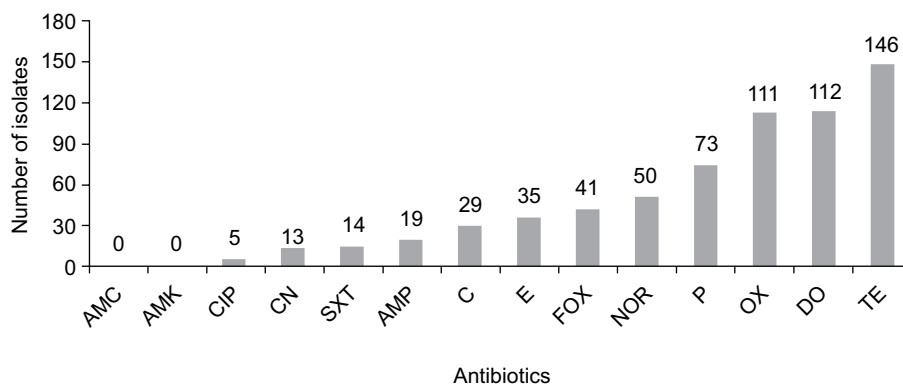
All the isolates (256) were susceptible to amoxicillin/clavulanic acid and amikacin. Fifty-seven percent (57.03%) of the isolates were resistant to tetracycline, 43.75% to doxycycline, 43.36% to oxacillin, and 28.52% to penicillin. The isolates showed low resistance to ampicillin (7.42%), trimethoprim/sulfamethoxazole (5.47%), gentamycin (5.00%), and ciprofloxacin (1.95%) (Figure 1).

**Table 1** Distribution of isolated coagulase-negative *Staphylococci* among the different sources

| CoNS source/<br>N (%) | <i>S. cohnii</i><br>1 (0.39) | <i>S. gallinarum</i><br>17 (6.64) | <i>S. haemolyticus</i><br>10 (3.91) | <i>S. lentus</i><br>92 (35.94) | <i>S. saprophyticus</i><br>5 (1.95) | <i>S. sciuri</i><br>120 (42.97) | <i>S. xylosus</i><br>11 (4.30) | Total (%)   |
|-----------------------|------------------------------|-----------------------------------|-------------------------------------|--------------------------------|-------------------------------------|---------------------------------|--------------------------------|-------------|
| AR                    | 1                            | 15                                | 9                                   | 22                             | 1                                   | 33                              | 5                              | 86 (33.59)  |
| BA                    | –                            | –                                 | 1                                   | 52                             | –                                   | 66                              | 6                              | 125 (48.83) |
| GR                    | –                            | 2                                 | –                                   | 18                             | 4                                   | 21                              | –                              | 45 (17.78)  |
| <b>Regional total</b> |                              |                                   |                                     |                                |                                     |                                 |                                |             |
| AR FW                 | –                            | 5                                 | 1                                   | 15                             | –                                   | 21                              | 1                              | 43 (16.80)  |
| BA FW                 | –                            | –                                 | –                                   | 14                             | –                                   | 19                              | –                              | 33 (12.90)  |
| GR FW                 | –                            | 2                                 | –                                   | 17                             | 4                                   | 15                              | –                              | 38 (11.33)  |
| <b>Total FW</b>       | –                            | 7                                 | 1                                   | 46                             | 4                                   | 55                              | 1                              | 114         |
| AR L                  | 1                            | 10                                | 8                                   | 7                              | 1                                   | 12                              | 4                              | 43 (16.80)  |
| BA L                  | –                            | –                                 | 1                                   | 38                             | –                                   | 47                              | 6                              | 92 (35.94)  |
| GR L                  | –                            | –                                 | –                                   | 1                              | –                                   | 6                               | –                              | 7 (2.65)    |
| <b>Total L</b>        | 1                            | 10                                | 9                                   | 46                             | 1                                   | 65                              | 10                             | 142         |

**Note:** “–” indicates no CoNS isolate.

**Abbreviations:** AR, Ashanti region; BA, Brong Ahafo region; CoNS, coagulase-negative *Staphylococcus*; FW, farm worker; GR, Greater Accra region; L, poultry bedding material; S., *Staphylococcus*.



**Figure 1** Resistance of CoNS isolates to selected antibiotics.

**Notes:** AMC, amoxicillin/clavulanic acid; AMK, amikacin; CIP, ciprofloxacin 5 µg; CN, gentamicin 10 µg; SXT, trimethoprim/sulfamethoxazole 25 µg; AMP, ampicillin 10 µg; C, chloramphenicol 30 µg; E, erythromycin 15 µg; FOX, ceftiofur 30 µg; NOR, norfloxacin 10 µg; P, benzyl penicillin 1 unit; OX, oxacillin 1 µg; DO, doxycycline 30 µg; TE, tetracycline 30 µg.  
**Abbreviation:** CoNS, coagulase-negative *Staphylococcus*.

**Table 2** Distribution of resistant coagulase-negative *Staphylococcus* spp. among selected antibiotics

|       | Antibiotics                   | P         | AMP       | OX         | FOX       | CIP      | NOR       | CN        | E         | TE         | DO         | C         | SXT       |
|-------|-------------------------------|-----------|-----------|------------|-----------|----------|-----------|-----------|-----------|------------|------------|-----------|-----------|
|       | <b>Resistant isolates (N)</b> | <b>73</b> | <b>19</b> | <b>111</b> | <b>41</b> | <b>5</b> | <b>50</b> | <b>13</b> | <b>35</b> | <b>146</b> | <b>112</b> | <b>29</b> | <b>14</b> |
| SP No | <b>Species</b>                |           |           |            |           |          |           |           |           |            |            |           |           |
| 1     | <i>S. cohnii</i>              | 1         | –         | 1          | 1         | –        | –         | –         | 1         | 1          | 1          | –         | –         |
| 17    | <i>S. gallinarum</i>          | 5         | –         | 17         | 15        | –        | –         | –         | 5         | 2          | 2          | 3         | –         |
| 10    | <i>S. haemolyticus</i>        | 10        | 6         | 3          | 2         | 1        | 1         | 2         | 1         | 4          | –          | –         | 1         |
| 92    | <i>S. lentus</i>              | 14        | –         | 51         | 3         | 3        | 43        | –         | 5         | 42         | 47         | 3         | 3         |
| 5     | <i>S. saprophyticus</i>       | 1         | –         | 2          | 2         | –        | –         | –         | –         | 5          | 3          | 4         | –         |
| 120   | <i>S. sciuri</i>              | 39        | 13        | 32         | 16        | 1        | 6         | 11        | 20        | 85         | 59         | 11        | 10        |
| 11    | <i>S. xylois</i>              | 3         | –         | 5          | 2         | –        | –         | –         | 3         | 7          | –          | 8         | –         |
|       | <b>Region</b>                 |           |           |            |           |          |           |           |           |            |            |           |           |
| 86    | AR                            | 32        | 9         | 50         | 26        | –        | 12        | 5         | 16        | 46         | 33         | 13        | 7         |
| 125   | BA                            | 31        | 4         | 41         | 7         | 3        | 29        | 4         | 12        | 76         | 58         | 10        | 5         |
| 45    | GR                            | 10        | 6         | 20         | 8         | 2        | 9         | 4         | 7         | 24         | 21         | 6         | 2         |
|       | <b>Litter/worker</b>          |           |           |            |           |          |           |           |           |            |            |           |           |
| 114   | Farm workers                  | 26        | 12        | 62         | 22        | 1        | 25        | 10        | 18        | 57         | 52         | 15        | 8         |
| 142   | Poultry litter                | 47        | 7         | 49         | 19        | 4        | 25        | 3         | 17        | 89         | 60         | 14        | 6         |
| 43    | FW-AR                         | 13        | 5         | 29         | 13        | –        | 8         | 5         | 8         | 23         | 19         | 5         | 4         |
| 33    | FW-BA                         | 4         | 2         | 15         | 3         | –        | 9         | 2         | 6         | 16         | 15         | 4         | 3         |
| 38    | FW-GR                         | 9         | 5         | 18         | 6         | 1        | 8         | 3         | 4         | 18         | 18         | 6         | 1         |
| 43    | Litter AR                     | 19        | 4         | 21         | 13        | –        | 4         | –         | 8         | 23         | 14         | 8         | 3         |
| 92    | Litter BA                     | 27        | 2         | 26         | 4         | 3        | 20        | 2         | 6         | 60         | 43         | 6         | 2         |
| 7     | Litter GR                     | 1         | 1         | 2          | 2         | 1        | 1         | 1         | 3         | 6          | 3          | –         | 1         |

**Notes:** “–” indicates no CoNS isolate. SP No, specimen number assigned to an isolate; P, benzyl penicillin 1 unit; AMP, ampicillin 10 µg; OX, oxacillin 1 µg; FOX, ceftiofur 30 µg; CIP, ciprofloxacin 5 µg; NOR, norfloxacin 10 µg; CN, gentamicin 10 µg; E, erythromycin 15 µg; TE, tetracycline 30 µg; DO, doxycycline 30 µg; C, chloramphenicol 30 µg; SXT, trimethoprim/sulfamethoxazole 25 µg.

**Abbreviations:** AR, Ashanti region; BA, Brong Ahafo region; GR, Greater Accra region; FW, farm worker.

## Distribution of resistant coagulase-negative *Staphylococcus* spp.

Each of the seven different coagulase-negative *Staphylococcus* spp. exhibited some level of resistance toward penicillin, oxacillin, ceftiofur, and tetracycline. All the different species were most susceptible toward ciprofloxacin. Isolates from all the three regions showed varying levels of resistance to the different antibiotics, with the exception of isolates from the Ashanti region which were totally susceptible to ciprofloxacin. Both sets of isolates from farm workers and poultry litter exhibited

various levels of resistance to the antibiotics. However, isolates from farm workers' samples in the Brong Ahafo and Ashanti regions and isolates from litter samples in the Ashanti regions did not exhibit any resistance toward ciprofloxacin. Also, none of the isolates obtained from litter samples in the Greater Accra region was resistant to chloramphenicol (Table 2).

## Multi-drug resistant CoNS

Forty-nine (19.14%) of the isolates exhibited multi-drug resistance (MDR) (simultaneous resistance to three or more anti-

otics) (Table 3).<sup>22</sup> Thirty isolates (61.22%) from farm workers were MDR isolates whilst 19 (38.78%) MDR isolates were from poultry litter. Twenty-three (46.94%) of the MDR strains were from samples from the Ashanti region, 15 (30.61%) from the Brong Ahafo region, and 11 (22.45%) from the Greater Accra region (Table 4). Among the *Staphylococcus* spp., 27 (55.1%) of the MDR isolates were *S. sciuri*, 9 (18.37%) *S. lentus*, 5 (10.20%) *S. gallinarum*, 3 (6.12%) *S. haemolyticus*, 2 (4.08%) isolates each were *S. saprophyticus* and *S. xyloso*, and 1 (2.04%) *S. cohnii*. Farm workers contributed 30 (61.22%) of the MDR Staphylococcal isolates whilst poultry litter had 19 (38.78%) of the MDR isolates (Figure 2).

## Discussion

The increasing rates of antibiotic resistance and MDR among pathogenic, non-pathogenic, commensals, and opportu-

nistic bacteria call for increased report on the distribution (prevalence) of these organisms and their antibiotic profiles.<sup>23</sup> Many *Staphylococcus* spp. are part of the normal bacterial flora in humans and animals.<sup>18</sup> Several of these commensal and non-pathogenic Staphylococci have been implicated in infections.<sup>5</sup> Many of such species have also been reported as multi-drug resistant, which has resulted in increased cost of treating infections and increased disease burden in both humans and animal husbandry.<sup>3,23</sup>

Seven different coagulase-negative *Staphylococcus* spp. including *S. sciuri*, *S. lentus*, *S. gallinarum*, *S. xyloso*, *S. haemolyticus*, *S. saprophyticus*, and *S. cohnii* were identified in both farm workers and poultry litter samples. The presence of different *Staphylococcus* spp. among poultry litter and farm workers has been reported by Simjee et al.<sup>24</sup> and Vadari et al.<sup>25</sup> The latter identified 19 different *Staphy-*

**Table 3** Antibiograms of isolated CoNS from different sources in the selected regions

| Resistance pattern (No of isolates)      | N  | AR |    | BA |    | GR |    |
|--|----|----|----|----|----|----|----|
|  |    | FW | PL | FW | PL | FW | PL |
| <b>No antibiotic (7)</b>                 | 7  | –  | –  | 5  | –  | 2  | –  |
| <b>One antibiotic (13)</b>               |    |    |    |    |    |    |    |
| C  | 2  | –  | –  | 1  | –  | 1  | –  |
| E  | 2  | –  | –  | 1  | –  | 1  | –  |
| P  | 4  | –  | –  | –  | 4  | –  | –  |
| TE                                       | 5  | –  | –  | –  | 3  | –  | 2  |
| <b>Two antibiotic combinations (187)</b> |    |    |    |    |    |    |    |
| CN-TE                                    | 1  | –  | –  | 1  | –  | –  | –  |
| DO-C                                     | 2  | –  | –  | 1  | –  | 1  | –  |
| E-TE                                     | 1  | –  | –  | –  | –  | –  | 1  |
| OX-C                                     | 1  | 1  | –  | –  | –  | –  | –  |
| OX-E                                     | 1  | 1  | –  | –  | –  | –  | –  |
| OX-FOX                                   | 11 | 5  | 6  | –  | –  | –  | –  |
| OX-NOR                                   | 36 | 5  | 3  | 5  | 16 | 7  | –  |
| OX-TE                                    | 3  | 2  | –  | –  | 1  | –  | –  |
| P-AMP                                    | 4  | –  | 3  | –  | 1  | –  | –  |
| P-DO                                     | 5  | 1  | –  | –  | 3  | 1  | –  |
| P-E                                      | 2  | –  | –  | –  | 2  | –  | –  |
| P-NOR                                    | 2  | –  | 1  | –  | 1  | –  | –  |
| P-OX                                     | 11 | 3  | 2  | 2  | 2  | 2  | –  |
| P-TE                                     | 22 | 3  | 8  | –  | 11 | –  | –  |
| TE-C                                     | 9  | –  | 2  | 1  | 4  | 2  | –  |
| TE-DO                                    | 76 | 9  | 8  | 8  | 37 | 12 | 2  |
| <b>Three antibiotic combinations (6)</b> |    |    |    |    |    |    |    |
| OX-CIP-NOR                               | 1  | –  | –  | –  | 1  | –  | –  |
| OX-FOX-E                                 | 1  | 1  | –  | –  | –  | –  | –  |
| OX-TE-DO                                 | 2  | 1  | –  | 1  | –  | –  | –  |
| P-AMP-OX                                 | 2  | –  | 1  | –  | –  | 1  | –  |
| <b>Four antibiotic combinations (6)</b>  |    |    |    |    |    |    |    |
| OX-E-TE-DO                               | 1  | 1  | –  | –  | –  | –  | –  |
| OX-FOX-E-C                               | 2  | –  | 2  | –  | –  | –  | –  |
| OX-FOX-TE-DO                             | 1  | –  | –  | –  | 1  | –  | –  |
| P-AMP-OX-CN                              | 2  | 1  | –  | 1  | –  | –  | –  |

(Continued)

Table 3 (Continued)

| Resistance pattern (No of isolates)      | N          | AR        |           | BA        |           | GR        |          |
|--|------------|-----------|-----------|-----------|-----------|-----------|----------|
|  |            | FW        | PL        | FW        | PL        | FW        | PL       |
| <b>Five antibiotic combinations (18)</b> |            |           |           |           |           |           |          |
| OX-CIP-NOR-TE-DO                         | 1          | –         | –         | –         | –         | 1         | –        |
| OX-E-TE-DO-C                             | 2          | –         | –         | 1         | –         | 1         | –        |
| OX-E-TE-DO-SXT                           | 3          | 1         | 1         | –         | 1         | –         | –        |
| OX-FOX-E-TE-DO                           | 1          | –         | –         | –         | –         | –         | 1        |
| OX-FOX-NOR-E-SXT                         | 1          | –         | –         | 1         | –         | –         | –        |
| OX-FOX-TE-DO-C                           | 2          | –         | 1         | –         | –         | 1         | –        |
| OX-NOR-TE-DO-SXT                         | 1          | –         | –         | 1         | –         | –         | –        |
| P-AMP-OX-FOX-CN                          | 3          | 1         | –         | –         | –         | 2         | –        |
| P-AMP-OX-FOX-SXT                         | 1          | –         | –         | –         | –         | 1         | –        |
| P-OX-FOX-E-C                             | 3          | –         | 2         | –         | 1         | –         | –        |
| <b>Six antibiotic combinations (11)</b>  |            |           |           |           |           |           |          |
| NOR-E-TE-DO-C-SXT                        | 1          | 1         | –         | –         | –         | –         | –        |
| OX-CIP-NOR-TE-DO-C                       | 1          | –         | –         | –         | 1         | –         | –        |
| OX-FOX-CN-E-TE-DO                        | 1          | –         | –         | –         | 1         | –         | –        |
| OX-FOX-NOR-E-TE-DO                       | 1          | –         | –         | 1         | –         | –         | –        |
| P-AMP-OX-E-TE-DO                         | 1          | –         | –         | 1         | –         | –         | –        |
| P-AMP-OX-FOX-CN-C                        | 1          | 1         | –         | –         | –         | –         | –        |
| P-AMP-OX-FOX-CN-E                        | 1          | –         | –         | –         | –         | 1         | –        |
| P-OX-E-TE-DO-SXT                         | 1          | –         | 1         | –         | –         | –         | –        |
| P-OX-FOX-E-TE-DO                         | 3          | 1         | 1         | –         | –         | 1         | –        |
| <b>Seven antibiotic combinations (4)</b> |            |           |           |           |           |           |          |
| OX-FOX-E-TE-DO-C-SXT                     | 1          | –         | 1         | –         | –         | –         | –        |
| OX-FOX-NOR-E-TE-DO-SXT                   | 2          | 1         | –         | 1         | –         | –         | –        |
| P-AMP-OX-FOX-CN-TE-DO                    | 1          | 1         | –         | –         | –         | –         | –        |
| <b>Eight antibiotic combinations (2)</b> |            |           |           |           |           |           |          |
| OX-FOX-NOR-E-TE-DO-C-SXT                 | 1          | 1         | –         | –         | –         | –         | –        |
| P-AMP-OX-FOX-CN-TE-DO-C                  | 1          | 1         | –         | –         | –         | –         | –        |
| <b>Nine antibiotic combinations (2)</b>  |            |           |           |           |           |           |          |
| P-AMP-OX-FOX-CIP-NOR-CN-E-SXT            | 2          | –         | –         | –         | 1         | –         | 1        |
| <b>Total number of isolates</b>          | <b>256</b> | <b>43</b> | <b>43</b> | <b>33</b> | <b>92</b> | <b>38</b> | <b>7</b> |

**Notes:** “–” indicates no CoNS isolated or identified. C, chloramphenicol; E, erythromycin; P, benzyl penicillin; TE, tetracycline; DO, doxycycline; OX, oxacillin; FOX, cefoxitin; NOR, norfloxacin; AMP, ampicillin; CIP, ciprofloxacin; CN, gentamicin; SXT, trimethoprim/sulfamethoxazole.

**Abbreviations:** CoNS, coagulase-negative *Staphylococcus*; AR, Ashanti region; BA, Brong Ahafo region; GR, Greater Accra region; FW, farm worker; PL, poultry litter.

*lococcus* spp. from poultry litter using molecular techniques (16s rDNA). The species included all the species identified in this study, with the exception of *S. gallinarum*. The low number of species identified in this study compared to that of Vadari et al.<sup>25</sup> could be due to the identification method used. Molecular techniques detect the presence of both viable and non-viable organisms, whilst only viable organisms are identified using morphological and biochemical means.

In the report by Simjee et al.,<sup>24</sup> 38% of the coagulase-negative *Staphylococcus* spp. were *S. sciuri*, whereas *S. lentus* and *S. xylosus* constituted 21% and 14%, respectively. The prevalence of *S. sciuri* among the farms in this study was 44%, whilst *S. lentus* and *S. xylosus* were 37% and 4%, respectively. *Staphylococcus simulans* was not found in this current study but was detected by Simjee et al.<sup>24</sup> On the other hand, *Staphylococcus* spp. including *S. cohnii*, *S. saprophyti-*

*cus*, and *S. gallinarum* were not identified and reported by Simjee et al.<sup>24</sup> The differences in the *Staphylococcus* spp. identified could be due to the different bacterial flora within the study areas.<sup>26</sup>

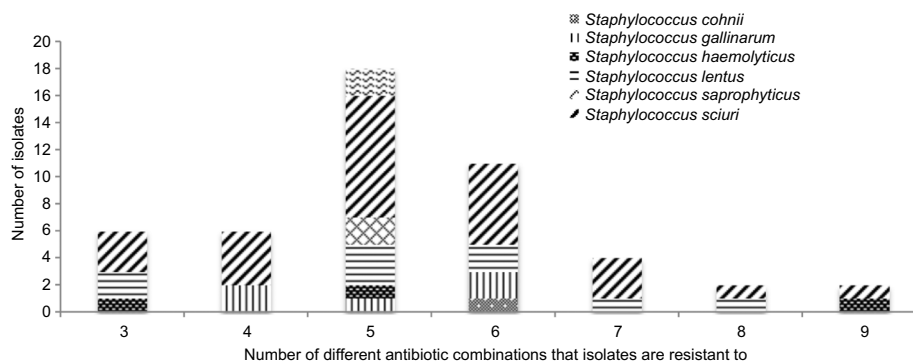
There have been reports of increasing resistance of *Staphylococcus* spp. to several essential antibiotics over the past 30 years<sup>26–28</sup> in different countries including Ghana.<sup>29</sup> In this study, coagulase-negative *Staphylococcus* spp. equally showed a high level of resistance mostly to tetracycline (57%), doxycycline (44%), and penicillin (28%). However, the resistance observed toward penicillin was less than the number Lerbech et al.<sup>29</sup> reported, with 98% CoNS isolates from humans being resistant to penicillin. The high resistance of CoNS to penicillin reported by Lerbech et al.<sup>29</sup> could be due to the fact that all the isolates were obtained from patients, and such isolates have been found to be highly

**Table 4** Distribution of multi-drug resistant CoNS strains against selected antibiotics

| Antibiotics                           | P         | AMP       | OX        | FOX       | CIP      | NOR       | CN        | E         | TE        | DO        | C         | SXT       |
|---------------------------------------|-----------|-----------|-----------|-----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| <b>Total isolates (N)</b>             | <b>22</b> | <b>15</b> | <b>48</b> | <b>30</b> | <b>5</b> | <b>12</b> | <b>12</b> | <b>29</b> | <b>29</b> | <b>29</b> | <b>15</b> | <b>14</b> |
| <b>TI Region</b>                      |           |           |           |           |          |           |           |           |           |           |           |           |
| 23 Ashanti                            | 11        | 6         | 22        | 15        | 0        | 3         | 5         | 15        | 14        | 14        | 10        | 7         |
| 15 Brong Ahafo                        | 4         | 3         | 15        | 7         | 3        | 7         | 3         | 9         | 10        | 10        | 3         | 5         |
| 11 Greater Accra                      | 7         | 6         | 11        | 8         | 2        | 2         | 4         | 5         | 5         | 5         | 2         | 2         |
| <b>Species</b>                        |           |           |           |           |          |           |           |           |           |           |           |           |
| 1 <i>Staphylococcus cohnii</i>        | 1         | 0         | 1         | 1         | 0        | 0         | 0         | 1         | 1         | 1         | 0         | 0         |
| 5 <i>Staphylococcus gallinarum</i>    | 3         | 0         | 5         | 5         | 0        | 0         | 0         | 5         | 2         | 2         | 3         | 0         |
| 3 <i>Staphylococcus haemolyticus</i>  | 3         | 3         | 3         | 2         | 1        | 1         | 2         | 1         | 0         | 0         | 0         | 1         |
| 9 <i>Staphylococcus lentus</i>        | 1         | 0         | 9         | 3         | 3        | 5         | 0         | 5         | 8         | 8         | 3         | 3         |
| 2 <i>Staphylococcus saprophyticus</i> | 0         | 0         | 2         | 2         | 0        | 0         | 0         | 0         | 2         | 2         | 2         | 0         |
| 27 <i>Staphylococcus sciuri</i>       | 12        | 12        | 26        | 15        | 1        | 6         | 10        | 15        | 16        | 16        | 5         | 10        |
| 2 <i>Staphylococcus xylosum</i>       | 2         | 0         | 2         | 2         | 0        | 0         | 0         | 2         | 0         | 0         | 2         | 0         |
| <b>Litter or worker</b>               |           |           |           |           |          |           |           |           |           |           |           |           |
| 30 Farm workers                       | 14        | 12        | 29        | 17        | 1        | 8         | 9         | 15        | 19        | 19        | 7         | 8         |
| 19 Poultry litter                     | 8         | 3         | 19        | 13        | 4        | 4         | 3         | 14        | 10        | 10        | 8         | 6         |
| 13 FW-AR                              | 6         | 5         | 12        | 8         | 0        | 3         | 5         | 7         | 9         | 9         | 4         | 4         |
| 8 FW-BA                               | 2         | 2         | 8         | 3         | 0        | 4         | 1         | 5         | 6         | 6         | 1         | 3         |
| 9 FW-GR                               | 6         | 5         | 9         | 6         | 1        | 1         | 3         | 3         | 4         | 4         | 2         | 1         |
| 10 Litter AR                          | 5         | 1         | 10        | 7         | 0        | 0         | 0         | 8         | 5         | 5         | 6         | 3         |
| 7 Litter BA                           | 2         | 1         | 7         | 4         | 3        | 3         | 2         | 4         | 4         | 4         | 2         | 2         |
| 2 Litter GR                           | 1         | 1         | 2         | 2         | 1        | 1         | 1         | 2         | 1         | 1         | 0         | 1         |

**Notes:** TI, total isolates; P, benzyl penicillin 1 unit; AMP, ampicillin 10 µg; OX, oxacillin 1 µg; FOX, cefoxitin 30 µg; CIP, ciprofloxacin 5 µg; NOR, norfloxacin 10 µg; CN, gentamicin 10 µg; E, erythromycin 15 µg; TE, tetracycline 30 µg; DO, doxycycline 30 µg; C, chloramphenicol 30 µg; SXT, trimethoprim/sulfamethoxazole 25 µg.

**Abbreviations:** CoNS, coagulase-negative *Staphylococcus*; AR, Ashanti region; BA, Brong Ahafo region; GR, Greater Accra region; FW, farm worker.

**Figure 2** Multi-drug resistant patterns of coagulase-negative *Staphylococci* isolated from poultry farms in the selected regions.

resistant to beta-lactams.<sup>30</sup> Resistance of the isolates to tetracycline (57%) was almost similar to the 63% reported by Lerbech et al.<sup>29</sup>

All the isolates from this study were susceptible to amoxicillin/clavulanic acid and amikacin. This may be due to the fact that these two antibiotics are not part of the antimicrobial agents routinely employed in poultry production in Ghana,<sup>31</sup> hence, amoxicillin/clavulanic acid and amikacin preparations remain reserved antibiotics used for the treatment of *Staphylococcus* spp. infections.<sup>32</sup>

Forty-nine (19.14%) of the CoNS isolated were multi-drug resistant with the highest resistance to tetracycline (59.18%), doxycycline (59.18%), and erythromycin (57.14%). Suleiman et al.<sup>33</sup> reported 65% MDR *Staphylococci* isolates in a similar study in Nigeria. The high level of MDR *Staphylococci* could be due to the misuse of antibiotics in the poultry industry in many developing countries, including Ghana<sup>13</sup> and Nigeria.<sup>34</sup> Coagulase-negative *Staphylococci* are also known to form biofilms and this reduces the effect of antimicrobial agents against them.<sup>26,27</sup>

The majority (61%) of the MDR CoNS isolates were isolated from humans including farm workers, owners, and managers, whereas the bedding material had 39% of the MDR isolates. These findings could be due to the fact that farm workers are more exposed to MDR *Staphylococcus* strains because of the direct contact or interactions between humans and animals. These findings confirm the reports by Lerbech et al.,<sup>29</sup> Feglo et al.,<sup>35</sup> Obeng-Nkrumah et al.,<sup>36</sup> and Newman et al.,<sup>37</sup> which showed a high level of antibiotic resistance among humans in Ghana.

## Conclusion

Coagulase-negative Staphylococci (256 isolates) from seven different species were identified and they exhibited varying levels of resistance to the selected antibiotics, with 49 multi-drug resistant isolates, and over 60% of these MDR *Staphylococcus* strains were from human samples. These call for increased surveillance measures and monitoring of antibiotic use in both animal husbandry and in humans in Ghana.

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## Disclosure

The authors report no conflicts of interest in this work.

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