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**Shivakumar Rekhyanaik**
Ph.D. Scholar, Department of Veterinary Microbiology, Karnataka Veterinary Animal and Fisheries Sciences University, Veterinary College, Bengaluru, Karnataka, India

**Wilfred Ruban**
Associate Professor and Head, Department of Livestock Products Technology, Karnataka Veterinary Animal and Fisheries Sciences University, Veterinary College, Bengaluru, Karnataka, India

**Srikrishna Isloor**
Professor, Department of Veterinary Microbiology, Karnataka Veterinary Animal and Fisheries Sciences University, Veterinary College, Bengaluru, Karnataka, India

**Ahlen IG Barry**
Junior Research Fellow, Department of Livestock Products Technology, Karnataka Veterinary Animal and Fisheries Sciences University, Veterinary College, Bengaluru, Karnataka, India

**Sharada Ramakrishna**
Associate Professor, Department of Veterinary Microbiology, Karnataka Veterinary Animal and Fisheries Sciences University, Veterinary College, Bengaluru, Karnataka, India

**Shivappa Nayaka**
Ph.D. scholar, Department of Poultry Science, Karnataka Veterinary Animal and Fisheries Sciences University, Veterinary College, Bengaluru, Karnataka, India

**Corresponding Author:**
**Shivakumar Rekhyanaik**
Ph.D. Scholar, Department of Veterinary Microbiology, Karnataka Veterinary Animal and Fisheries Sciences University, Veterinary College, Bengaluru, Karnataka, India

# Antibiogram pattern of AMR *E. coli* from feed, water, boot socks and hand swab samples in pig farms located in and around Bengaluru, India

**Shivakumar Rekhyanaik, Wilfred Ruban, Srikrishna Isloor, Ahlen IG Barry, Sharada Ramakrishna and Shivappa Nayaka**

## Abstract
A study was carried out to evaluate the antimicrobial susceptibility pattern of *E. coli* isolated from feed, water, boot socks and human hand swabs of animal attender from 10 organized pig farms located in and around Bengaluru, Karnataka, India. All Samples from 10 organized pig farms (3 feed samples, 3 water samples, 3 Hand swabs and 2 samples of boot socks dust from inside the farm and one outside of the farm were collected from each farm) were collected and were used for isolation and characterization of *E. coli* based on biochemical methods. *E. coli* was isolated from all the samples. A total of 18 *E. coli* isolates obtained from feed samples, 12 *E. coli* isolates obtained from water samples, 25 *E. coli* isolates obtained from boot socks and 14 *E. coli* isolates obtained from hand swabs and were screened for antimicrobial susceptibility testing based on disc diffusion assay against 13 commonly used antimicrobials in human and veterinary medicine. Out of the of 18 *E. coli* isolates obtained from different feed samples revealed highest resistance to Doxycycline (94.44%) whereas the least resistance was observed in gentamicin, neomycin and cefpodoxime + clavulanic acid (5.56%), Among the 12 *E. coli* isolates from water samples 100 percent resistance was observed for Ciprofloxacin and the least resistance was observed in neomycin, chloramphenicol and amoxicillin each at 8.33 percent. Out of the 25 *E. coli* isolates recovered from Boot sock dust, 92 percent exhibited resistance towards doxycycline (Tetracyclines) and the least resistance was observed in amoxicillin (12%). Out of the 14 *E. coli* isolates recovered from animal attender hand swabs, 100 percent resistance was observed in Doxycycline and least resistance was observed in ceftazidime + clavulanic acid and Cefotaxime + clavulanic acid (14.29%) as well as amoxicillin (7.14%). However, none of the isolates recovered from water samples and animal attender hand swabs exhibited resistance towards gentamicin, amikacin, neomycin, imipenem and meropenem.
The phenotypic characterization confirmed the presence of Extended spectrum β-lactamase producing *E. coli* was highest in water samples (41.67%), followed by feed samples (27.78%), boot sock dust (20%) and the lowest ESBL presence was recorded in animal attender hand swabs (16.67%). The presence of Multidrug resistant (MDR) was highest in water samples (91.66%) followed by feed samples (88.88%), animal attender hand swab (85.71%), and boot sock dust (80%) being resistant to one antimicrobial agent in three or more antimicrobial classes. The study clearly indicated higher presence of multidrug resistant *E. coli* in feed, water, boot socks and human hand swabs of animal attender indicating its public health significance and potential of such samples being a major source in dissemination of AMR *E. coli* in the entire pork production chain.

**Keywords:** AMR, *E. coli*, adult pigs, multidrug resistant, antibiotics

## Introduction
Antimicrobial resistance (AMR) is one of the major and growing public health threat worldwide and indiscriminate use of antimicrobials in food-producing animals for prophylaxis and as growth promoter has been documented to be the primary reason in emergence and spread of AMR in animal production system (Lim *et al*., 2014) [15]. In developing countries like India, rearing of livestock especially pigs and poultry, which plays an important role in improving the socio-economic status of the poor and marginal farmers has been practiced as family subsistence system or under backyard system with lower inputs in terms of feed and lower antimicrobial usage (Vinodh Kumar *et al*., 2019) [24]. In the recent past, there has been a significant transformation in pig farming in India from backyard system of rearing to small/medium or larger commercial farms owing to the high demand for animal protein. However, with the increase in scale of productivity, animals have been exposed to various infections and hence use of antibiotics is often regarded as the simplest way to maintain healthy and productive animals (Manyi-Loh *et al*., 2018) [18]. In addition, over the counter availability of antimicrobials have also contributed to the extensive use of antimicrobials by

the livestock owners, which in turn has contributed to the emergence of AMR in livestock production system (Kotwani *et al*., 2021) [10].

Globally, livestock are reared in farm environment, which is one of the most ignored components studied when it comes to antibiotic resistance evolution, transmission and persistence, presumably due to perceived lower importance (Jadeja and Worrich, 2022). The transmission of AMR from the animals to its environment though manure may contribute to the increase in the drug resistance in the environment and may play a vital role in transfer of resistance to humans (Duse *et al*., 2016).

In India, livestock and poultry sectors significantly contribute to the rural economy. Nevertheless, these sectors are also crippled by various health related issues including the AMR. However, such studies on AMR in entire production system taking into consideration the human animal interface have been well documented in poultry unlike in livestock. This could be attributed to its highly intensive production system with evidence of antimicrobial use (AMU) (Brower *et al*., 2017) [3]. Globally, including India, among livestock, the pork sector is up coming and is also reportedly being affected by AMR. However to date, most studies of AMR in pigs have only involved cross-sectional observations taken at specific periods of production, with the studied farms having a lack of availability of data about AMU. In general, no systemic studies have been carried out to document the occurrence of AMR in pork production system as a whole. Hence, a more holistic approach for monitoring the occurrence of AMR at different points of the production cycle, antimicrobial usage (AMU) and examination of environmental samples of the farm should be carried out to have a better understanding of the dynamics of AMR in the entire pork production system to formulate effective control strategies. In Bengaluru, a cosmopolitan city, pork is gaining popularity and the pig farming is also picking up in and around the city. Hence, the present study has been carried out to evaluate the occurrence of antibiotic resistance in pork production system with *E. coli* as the indictor organism in commercial pig farms in and around Bengaluru, Karnataka, India.

## Materials and Methods

The present study was carried out in 10 commercial pig farms located in and around Bengaluru, Karnataka, India. All Samples from 10 organized pig farms (3 feed samples, 3 water samples, 3 Hand swabs and 2 samples of boot socks dust from inside the farm and one outside of the farm were collected from each farm) were collected and were used for isolation and characterization of *E. coli* based on biochemical methods. using sterile containers and were transported to the laboratory for isolation and characterization of *E. coli*. Isolation of *E. coli* was done as per the standard procedure (Quinn *et al*. 2002) [20]. In brief, samples were pre-enriched in Brain heart infusion and enriched for 8-10 hr at 37ºC. The enriched samples were streaked onto Eosin Methylene Blue and MacConkey agar (Himedia, India) and isolates were gram stained and identified up to species level using standard bacteriological techniques, including colony morphology on MacConkey and Eosin Methylene Blue Agar, oxidase, catalase, urease and indole tests as per Quinn *et al*. (2002) [20]. The *E. coli* isolates were then subjected to antibiotic susceptibility testing of isolates to a panel of 13 antimicrobial agents (Gentamicin (GEN=10µg) Amikacin (AK=30µg), Neomycin (N=10µg), Ciprofloxacin (CIP=5µg), Enrofloxacin (EX=5µg), Doxycycline (DO=30µg), Trimethoprim-sulfamethoxazole {COT=25(23.75/1.25 µg)}, Chloramphenicol (C=30 µg), Ampicillin (AMP=10 µg), Amoxicillin + Clavulanic acid (AMC=20/10 µg), Imipenem (IMP=), Meropenem (MRP=), Cefotaxime (CTX=30 µg), Cefotaxime + Clavulanic acid (CEC=30/10 µg)) using the Kirby-Bauer method (disc diffusion method) (Bauer *et al*. 1966). Interpretation of the results was carried out as per European committee on Antimicrobial Susceptibility Testing (EUCAST, 2020) [8] and Clinical and Laboratory Standard Institute (CLSI, 2018) [5] wherever the EUCAST breakpoints were not available. There are no breakpoints for Doxycycline, and instead the breakpoint for Tetracycline were used. The standard reference strain of *E. coli* ATCC 25922, was used as the quality control strain. Intermediate isolates were grouped with resistant isolates. Resistance profiles were generated and isolates were classified as MDR if they showed resistance to one antimicrobial agent in three or more antimicrobial classes (Magiorakos *et al*., 2012) [16].

## Result and Discussion

Antimicrobial susceptibility pattern of *E. coli* isolates obtained from different feed samples revealed highest resistance to Doxycycline (94.44%) followed by cotrimoxazole and ampicillin (72.22%), Enrofloxacin (61.11%), Cefotaxime + clavulanic acid (44.44%), ceftazidime + clavulanic acid (38.89%), ciprofloxacin, chloramphenicol and amoxicillin 27.78 percent each, whereas the least resistance was observed in gentamicin, neomycin and cefpodoxime + clavulanic acid (5.56%) and all the *E. coli* isolates tested were susceptible to amikacin, imipenem and meropenem.

Among the *E. coli* isolates from water samples 100 percent resistance was observed for Ciprofloxacin, followed by Doxycycline (91.67%), Enrofloxacin and ampicillin each at 83.33 percent, cotrimoxazole (58.33%), ceftazidime + clavulanic acid (50%), cefpodoxime + clavulanic acid (41.67%), Cefotaxime + clavulanic acid (33.33%) and the least resistance was observed in neomycin, chloramphenicol and amoxicillin each at 8.33 percent. It was also observed that all the *E. coli* isolates tested were susceptible gentamicin, amikacin, imipenem and meropenem.

Out of the 25 *E. coli* isolates recovered from Boot sock dust, 92 percent exhibited resistance towards doxycycline (Tetracyclines) followed by Enrofloxacin and ciprofloxacin (76%), ceftazidime + clavulanic acid (68%), ampicillin (48%), cotrimoxazole (44%), Cefotaxime + clavulanic acid (40%), chloramphenicol (20%), cefpodoxime + clavulanic acid (16%) and amoxicillin (12%).

Out of the 14 *E. coli* isolates recovered from animal attender hand swabs, 100 percent resistance was observed in Doxycycline followed by Ciprofloxacin and ampicillin 85.71percent each, Enrofloxacin (71.43%), cotrimoxazole (57.14%), chloramphenicol (50%), cefpodoxime + clavulanic acid (21.43%) and least resistance was observed in ceftazidime + clavulanic acid and Cefotaxime + clavulanic acid (14.29%) as well as amoxicillin (7.14%). However, none of the isolates recovered from water samples and animal attender hand swabs exhibited resistance towards gentamicin, amikacin, neomycin, imipenem and meropenem.

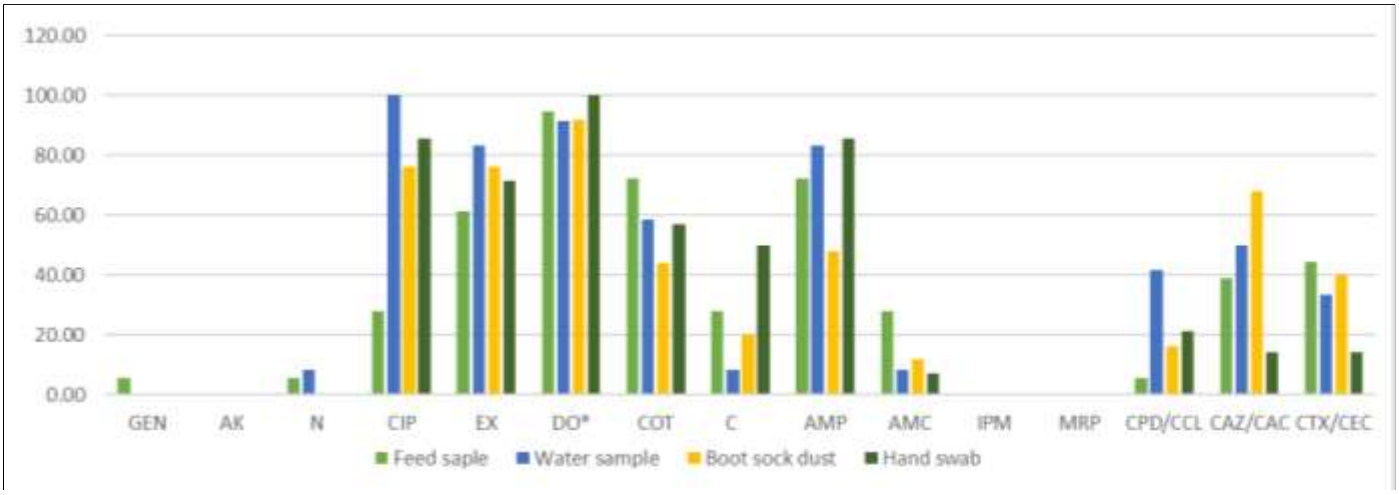
The results of antimicrobial susceptibility testing of *E. coli* from host related and environmental samples from pork production system indicated that majority of the isolates showed resistance to tetracycline (93.14-95.19%) followed by fluoroquinolones group *viz*., Enrofloxacin, ciprofloxacin and Ampicillin. The results of the study are in concurrence with the findings of Abubakar *et al*. (2019) in piglets of Pretoria, Van Den Bogaard *et al*. (2000) [23] in Netherland who observed higher resistance towards tetracycline and Lim *et al*. (2014) in Korea who reported that the rate of resistance of *E. coli* from pigs to tetracycline was the highest, followed by Ampicillin. In similar lines, higher resistance towards tetracycline, quinolones, Ampicillin, sulfonamides have been documented by Mandakini *et al*. (2020) [17] from pig population of organized and unorganized farms of Mizoram, Tamta *et al*. (2020) in fecal samples of piglets and pig farm workers of selected organized farms of India, Strom *et al*. (2017) in small-scale urban pig farming in a lower middle-income country, Kidsley *et al*. (2018) in healthy pigs in Australia and Zhang *et al*. (2017) in free range pigs in China. The higher resistance observed in this study may be attributed to the wider use of tetracyclines and fluoroquinolones in the treatment of commonly observed pig diseases, their ease of availability over the counter without any prescription and its broad spectrum of activity.

The isolates in the present study revealed complete sensitivity to carbapenem class of antibiotics (Imipenem and meropenem) followed by aminoglycoside group (Gentamicin, Amikacin and Neomycin, Chloramphenicol and Amoxicillin and Clavulanic acid. Similar observations have been documented by Kyung-Hyo *et al*. (2020) [11], who have reported that ban on use of antibiotics as growth promoter in piglets caused a decrease in resistance to gentamicin, neomycin, ciprofloxacin, norfloxacin and amoxicillin/clavulanic acid in *E. coli* isolated from weaned piglets in Korea. This is supported by Liu *et al*. (2016) who opined that AMR is dependent on the level of AMU and in the present study aminoglycosides and chloramphenicol were seldom used in treatment of pigs substantiates its lower resistance or higher sensitivity.

In the present study, the presence of Multidrug resistant (MDR) was highest in water samples (91.66%) followed by feed samples (88.88%), animal attender hand swab (85.71%), and boot sock dust (80%) being resistant to one antimicrobial agent in three or more antimicrobial classes. The results are in agreement with the findings of Abdalla *et al*. (2022) in *E. coli* from intensive pig production (73.0%), Abdalla *et al*. (2021) in *E. coli* from an Intensive Pig Production System in South Africa (71.2%), Kallau *et al*. (2018) in Escherichia coli at pig farm in Indonesia (57.3%) and Lugsomya *et al*. (2018) in pig farms in Thailand (73.3%). Similar findings have been documented by Sanjukta *et al*. (2019) in apparently health pigs in North East (90%), Kyung-Hyo *et al*. (2020) [11] in weaned piglets (95%), Momtaz *et al*. (2012) in commercial chicken meat (64.91%) and Brower *et al*. (2017) [3] in layer chicken (60%).

**Table 1:** Antibiotic Resistance pattern of AMR *E. coli* isolates from Feed, Water, Boot sock dust and Hand Swab Samples

| Group of Antimicrobials | Antibiotic discs used | Antibiotic concentration (mcg) | Feed (n=18) | Water (n=12) | Boot sock (n=25) | Hand swab (n=14) |
|---|---|---|---|---|---|---|
| Aminoglycosides | GEN | 10 | 5.56 | 0.00 | 0.00 | 0.00 |
| | AK | 30 | 0.00 | 0.00 | 0.00 | 0.00 |
| | N | 30 | 5.56 | 8.33 | 0.00 | 0.00 |
| Fluoroquinolones | CIP | 5 | 27.78 | 100.00 | 76.00 | 85.71 |
| | EX | 5 | 61.11 | 83.33 | 76.00 | 71.43 |
| Tetracycline | DO* | 30 | 94.44 | 91.67 | 92.00 | 100.00 |
| Folate pathway inhibitors | COT | 30 | 72.22 | 58.33 | 44.00 | 57.14 |
| Phenicols | C | 30 | 27.78 | 8.33 | 20.00 | 50.00 |
| Penicillin/ β lactamase inhibitors | AMP | 10 | 72.22 | 83.33 | 48.00 | 85.71 |
| | AMC | 20/10 | 27.78 | 8.33 | 12.00 | 7.14 |
| Carbapenems | IPM | 2 | 0.00 | 0.00 | 0.00 | 0.00 |
| | MRP | 30 | 0.00 | 0.00 | 0.00 | 0.00 |
| Extended-spectrum β-lactamase inhibitors | CPD/CCL | 30/10 | 5.56 | 41.67 | 16.00 | 21.43 |
| | CAZ/CAC | 30/10 | 38.89 | 50.00 | 68.00 | 14.29 |
| | CTX/CEC | 30/10 | 44.44 | 33.33 | 40.00 | 14.29 |




**GEN:** Gentamycin, **AK:** Amikacin, **N**: Neomycin, **CIP**: Ciprofloxacin; **EX**: Enrofloxacin, **DO**: Doxycycline, **COT**: Cotrimoxazole, **C**: Chloramphenicol, **AMP**: Ampicillin, **AMC:** Amoxicillin, **IPM:** Imipenem, **MRP**: Meropenem, **CPD/CCL**: Cefpodoxime + clavulanic acid, **CAZ/CAC:** Ceftazidime + Clavulanic acid **CTX/CEC**: Cefotaxime + Clavulanic acid.

**Fig 1:** Antibiotic Resistance pattern of *E. coli* isolated from Feed, Water, Boot sock dust and Hand Swab Samples

## Conclusion

The results of the present study indicated that commensal *E. coli* from apparently healthy adult pigs were found to be resistant to antibiotics commonly used in human medicine and occurrence of resistance towards cephalosporins (ESBL) are of major concern as these isolates may be involved in dissemination of AMR to farm workers. In addition, presence of MDR *E. coli* in pigs indicates that animals are being exposed to varying environment and the resistance could have been environmentally acquired, which requires further investigation.

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