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Antimicrobial susceptibility pattern of *E. coli* isolated from healthy piglets in pig farms located in and around Bengaluru, India

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Abstract
A cross sectional study was carried out to evaluate the antimicrobial susceptibility pattern of *E. coli* isolated from healthy piglets from 10 organized pig farms located in and around Bengaluru, Karnataka, India. Fecal samples from 40piglets (4 piglets 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 106 *E. coli* isolates obtained from healthy piglets and were screened for antimicrobial susceptibility testing based on disc diffusion assay against 13commonly used antimicrobials in human and veterinary medicine. The results revealed that majority of the isolates were resistant to Tetracycline (94.34%) followed by fluoroquinolones group viz., Enrofloxacin (89.62%), Ciprofloxacin (79.24%) and Ampicillin (60.38%). All the isolates in the present study were 100 per cent sensitive to carbapenem group of antibiotic tested (Imipenem and Meropenem). In the present study, it was evident that isolates from piglets were found to be sensitive to aminoglycoside group (Gentamicin-94.34%, Amikacin-990.6% and Neomycin-97.17%), Chloramphenicol (74.53%) and Amoxicillin and Clavulanic acid (82.08%). The phenotypic characterization confirmed the presence of extended spectrum β-lactamase *E. coli* at 33.02 per cent. In all, 83.02 per cent (88/106) of *E. coli* isolates were Multidrug resistant (MDR) were being resistant to one antimicrobial agent in three or more antimicrobial classes. The study clearly indicated higher presence of multidrug resistant *E. coli* in healthy piglets indicating its public health significance and potential of such piglets being a major source in dissemination of AMR *E. coli* in the entire pork production chain.

Keywords: AMR, *E. coli*, piglets, 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) [22]. 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].
Escherichia coli is an abundant commensal enteric bacterium in intestinal tract of domestic animals and has been used as an indicator organism for studying AMR in the food chain as it is found in all the compartments of the production system. It has been documented that commensal bacteria in gastrointestinal tract of both animals and humans are considered to be good indicators of AMR as they are exposed to selection pressure driven by any antimicrobial use in the host (Blake *et al*., 2003) [2]. However, the role of these commensal bacteria in carriage of AMR in healthy piglets has been neglected. Hence, the present study has been

carried out to evaluate the occurrence of antibiotic resistance in fecal *E. coli* isolates from healthy piglets in commercial pig farms in and around Bengaluru, Karnataka, India.

Materials and Methods

The present cross sectional study was carried out in 10 commercial pig farms located in and around Bengaluru, Karnataka, India. A total of 40 piglets (4 each from one farm) were used in this study. Rectal swabs were taken from these piglets using sterile cotton swabs 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 37 ºC. 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(23.75/1.25µg)}, Chloramphenicol (C=30µg), Ampicillin (AMP=10µg), Amoxicillin + Clavulanic acid (AMC=20/10µg), Imipenem (IMP=), Meropenem (MRP=), Cefotaxime (CTX=30µg), Cefotaxime + Clavulanic acid (CEC=30/10µg)) 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 was 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

In the present study, *E. coli* was isolated from all the rectal samples from the healthy piglets from all the farms as *Escherichia coli* is a commensal flora in human and animal intestines (Dohmen *et al*., 2017) [7]. A total of 106 *E. coli* were isolated from 40 rectal samples and similar isolation have been carried out Lalzampuia *et al*. (2013) [14] who isolated 102 *E. coli* from 53 fecal samples from pigs in Mizoram and Lalruatdiki *et al*. (2018) [13] who isolated 867 *E. coli* from 228 fecal samples from pigs of Meghalaya and Assam. The isolation rate of *E. coli* varies with the type of samples, media used, isolation protocol and person to person variation. In this study, 3-4 suspected colonies were randomly picked and used for identification based on biochemical characterization.

Antibiotics especially with broad spectrum of activity are extensively used in intensive pig production system for effective disease control, to ensure good health and welfare of the animals, which results in emergence of AMR (Diana *et al*., 2017) [6]. The results of antimicrobial susceptibility testing of the present study indicated that majority of the isolates showed resistance to tetracycline (94.34%) followed by fluoroquinolones group *viz*., Enrofloxacin (89.62%), ciprofloxacin (79.24%) and Ampicillin (60.38%). The results of the study are in concurrence with the findings of Abubakar *et al*. (2019) [1] in piglets (73.1%) of Pretoria, Van Den Bogaard *et al*. (2000) [21] in Netherland who observed higher resistance towards tetracycline. In similar lines, Cho *et al*. (2006) [4] and Lim *et al*. (2014) [15] in Korea reported that the rate of resistance of *E. coli* from pigs to Tetracycline was the highest (97.8%), followed by Ampicillin (89.1%). 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 drug over the counter without any prescription and broad spectrum of activity.

Table 1: Antibiotic susceptibility pattern of *E. coli isolated* from healthy piglets from pig farms in and around Bengaluru

Class of antibiotic	Name of antibiotics	*E. coli* isolates from piglets (n=106)	
		Sensitive	Resistant
Aminoglycosides	GEN	100 (94.34)	6 (5.66)
	AK	105 (99.06)	1 (0.94)
	N	103 (97.17)	3 (2.83)
Fluoroquinolones	CIP	22 (20.75)	84 (79.24)
	EX	11 (10.38)	95 (89.62)
Tetracycline	DO	6 (5.66)	100 (94.34)
Folate pathway inhibitors	COT	62 (58.49)	44 (41.51)
Phenicols	C	79(74.53)	27(25.47)
Penicillin/β-lactamase inhibitors	AMP	42(39.62)	64(60.38)
	AMC	87(82.08)	19(17.92)
Carbapenems	IMP	106(100)	-
	MRP	106(100)	-
Extended-spectrum β-lactamase	CTX-CEC	71(66.98)	35(33.02)

The isolates in the present study revealed complete sensitivity to carbapenem class of antibiotics (Imipenem and meropenem) followed by aminoglycoside group (Gentamicin-94.34%, Amikacin-99.06% and Neomycin-97.17%), chloramphenicol (74.53%) and Amoxicillin and clavulanic acid (82.08%). 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*. (2014) [15] who opined that AMR is dependent on the level of antimicrobial usage and in the present study farms aminoglycosides and chloramphenicol were seldom used in treatment of pigs substantiates its low resistance or higher sensitivity.

The prevalence of ESBL *E. coli* in the healthy piglets in this study was 33.02 per cent. Extended spectrum β-lactamases (ESBLs) have been reported worldwide, most frequently in *Enterobacteriaceae* (Lalak *et al*., 2016) [12]. Similar prevalence have been recorded in pigs and piglets by Dohmen *et al*. (2017) [7] in Netherland (24.7%) and Galina *et al*. (2021) [9] in Lativa (26.9%). However, contrary to the findings of this study Mandakini *et al*. (2020) [17] observed that 65.89 per cent of *E. coli* isolates from organized and unorganized pig farms in Meghalaya were ESBLs producer and VinodhKumar *et al*. (2019) [22] who reported 64 per cent prevalence of ESBL *E. coli* in organized pig farms across India. In this study we observed that none of the farms used cephalosporins for treatement of the pigs. Among the isolates screened 83.02 per cent (88/106) of the isolates were multidrug resistant (MDR) being resistant to one antimicrobial agent in three or more antimicrobial classes tested. Similarly higher rate of MDR E. coli have been reported by Sanjukta *et al*. (2012) in apparently health pigs in North East (90%), Kyung-Hyo *et al*. (2020) [11] in weaned piglets (95%), Momtaz *et al*. (2012) [19] in commercial chicken meat (64.91%) and Brower *et al*. (2017) [3] in layer chicken (60%).

Conclusion

The results of the present study indicated that commensal *E. coli* from apparently healthy piglets 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 piglets indicates that young animals are being exposed to varying environment and the resistance could have been environmentally acquired, which requires further investigation.

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