A cross sectional study on the prevalence of MDR Staphylococci and E. coli from livestock in Karnataka, India

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Abstract

In India, despite extensive studies on multi drug resistant bacterial pathogens in humans and animals, there is a wide gap in systematic surveillance in farming animals to have a clear vision on the prevalence of Antimicrobial Resistance (AMR). In this cross-sectional study, a total of 236 samples from livestock (cattle = 128), poultry = 45), swine = 2) and small ruminants = 61)) were collected during 2019-20 from Chikkaballapur district, Karnataka to identify and characterize multidrug resistant bacterial pathogens particularly *Staphylococcus* spp. and *E. coli*. The phenotypically identified isolates were confirmed by molecular screening, further antimicrobial susceptibility was determined by disc diffusion, broth microdilution and confirmatory tests (combined disc diffusion method). Resistant gene markers *mec*A and *mec*C genes were targeted in *Staphylococcus* spp. and extended spectrum beta lactamase (ESBL), AmpC type beta lactamase and carbapenem genes in *E. coli* isolates and screened by PCR. A total of 225 isolates recovered, 88% were *Staphylococcus* spp. and 47.5% were *E. coli* isolates. Overall prevalence of Multiple Drug resistance (MDR) was 20.35% and 35.71% in *Staphylococcus* spp. and *E. coli*, respectively. Methicillin resistant *Staphylococcus* was found in 12% of Staphylococci and 9% of *E. coli* isolates which carried ESBL/AmpC/New Delhi metallo β-lactamase (NDM) genes. This study provides an insight into the prevalence of AMR in *Staphylococci* and *E. coli* and their transmission potential to humans through food chain.

Introduction

One of the most important and significant discovery of 20th century was the magic bullet “Antibiotics” which revolutionized medical science and saved millions of lives (Aminov, 2010). Previously, antibiotics were used only for treating serious infections, later it was excessively used for preventing infections of surgical patients, protecting cancer patients, promoting growth, preventing diseases in livestock and other food animals (Ventola, 2015). Indiscriminate use of antibiotics was the major driver for resistance, which is a global public health threat (Laxminarayan et al. 2013). The factors such as unregulated sales, high disease burden, weak public health infrastructure, cheaper price and over the counter access of antibiotics paved the way for larger consumption of drugs (Laxminarayan et al. 2013). Continuous overuse has resulted in resistance to more than one antibiotic which led to emergence of Multiple Drug Resistant (MDR) pathogens, and later developed resistance to last-resort antibiotics. Regardless of Country’s income and advanced healthcare system, antibiotic ineffectiveness has raised as a major social threat (Fair et al. 2014). Bacteria are present everywhere, with the interconnected ecosystems and thus the Antimicrobial Resistance (AMR) problem is no longer limited to medical science alone. Effective collaboration among several sectors and one health approach is needed to address the issue in humans, animals, and environment.

India has highest bacterial disease burden in the world and thus depends on antibiotics (WHO, 2014). Also, India is the largest consumer of antibiotics especially for animal production alone the consumption was estimated to be 30 kg/km^2 (Ganguly et al. 2011, Vishnura et al. 2016). The intimate contact of the human beings with the livestock and common ecological niches they share, show that, these formidable pathogens are no longer restricted within human beings or animals. To understand the exact scenario of AMR in various sectors, a robust data without any ambiguity is critical and to achieve this goal, there must be a consensus guidelines focused on the sampling plan, methodologies, and interpretations need to be adopted. Considering the complex nature of the AMR problem, this strategically framed study was conducted as a part of the surveillance program aimed to characterize and understand the resistance determinants of *E. coli* and *Staphylococci* from different livestock.

Material And Methods

Study design

The different livestock species from unorganized animal herds were chosen following cluster sampling method in which Chikkaballapur district was selected as primary sampling unit. Within this district, four clusters designated as blocks were selected based on geographical distance (approximately 30 kilometers apart). Within each cluster, four epi units (village) were selected randomly (approximately 5-10 kilometers apart). In the second strata, the households (HH) were randomly selected based on the sample availability within each epi unit to collect 15 samples from all the animal species (cow/buffalo milk samples- 8, poultry cloacal swabs-3, sheep/goat rectal swabs-2, pig rectal swabs-2). Animal species was relaxed based on the availability and importance of animal domestication in that geographical area. The farmers were contacted before the study by key veterinary health care staff and informed consents were obtained from all the households.

Sample information sheet

A questionnaire was designed which contained the general information (Block name, village name, farmers name, contact number and date of sample collection), epidemiological metadata (latitude, longitude and disease outbreak history), sample source and nature (sample details from different food animals, animal handler details), management practices (rearing practices, feeding practices, milking practices, sanitary practices) and sample transport information (sample ID, date of transport) along with the consent for each sample was collected. The data was entered in M.S Excel software and blind folded data cleaning was done. The geographical indication coordinates were noted, and map (Fig.1) was prepared in QGIS software version 3.14.

Samples collected

As per study design out of 240, 236 samples (105 cow milk samples, 23 buffalo milk samples, 45 poultry cloacal swabs, 61 sheep/ goat rectal swabs and 2 pig rectal swabs) were collected from 200 households in sixteen epi units under four clusters of Chikkaballapur District, Karnataka (Chikkaballapur, Gauribidanur, Sidlaghatta, Chintamani) between January to December 2019 (Fig.1). The sample distribution details are annexed in Table 1.

Table 1
Isolation of *Staphylococcus* spp. and *E. coli* from different sampling sites

Block	Village	Households	Latitude	Longitude	Cow milk	Buffalo milk	Poultry rectal swab	Sheep/Goat rectal swab	Pig rectal swab	Total	*Staphyloco* spp
Chikkaballapur	Kalavara	9	13.416	77.668	6	2	3	4	0	15	6
	Manchanabale	9	13.442	77.831	7	1	3	4	0	15	2
	Thumukalahalli	12	13.376	77.803	6	2	3	4	0	15	9
	Gundalagurki	8	13.432	77.689	4	4	3	2	2	15	7
Subtotal										60	24
Gauribidanur	Haleupparahalli	10	13.592	77.448	4	4	0	4	0	12	8
	Hosuru	13	13.536	77.504	8	0	3	3	0	14	10
	Mudigere	15	13.561	77.468	5	3	3	4	0	15	7
	Kadalaveni	15	13.594	77.583	4	4	3	4	0	15	9
Subtotal										56	34
Sidlaghatta	Kakachokkondahalli	9	13.315	77.854	8	0	3	4	0	15	6
	Nagamangala	15	13.256	77.947	8	0	3	4	0	15	6
	Gattamaranahalli	15	13.258	77.858	8	0	3	4	0	15	7
	Jangamakote	15	13.31	77.839	7	1	3	4	0	15	8
Subtotal										60	27
Chintamani	Kendanahalli	10	13.332	78.079	7	1	3	4	0	15	6
	Thimmasandra	14	13.393	78.092	7	1	3	4	0	15	6
	Nayanahalli	15	13.412	78.041	8	0	3	4	0	15	7
	Konapalli	15	13.393	78.045	8	0	3	4	0	15	9
Subtotal										60	28
Grand total		200								236	113 (88.28

*value in parenthesis represents percentage.

Isolation, phenotypic and genotypic identification of bacterial isolates

All the collected samples were transported to the Bacterial epidemiology laboratory, ICAR-NIVEDI, Bangalore, India. For isolation of *Staphylococcus* spp., 128 milk samples were inoculated into Mannitol salt broth (MSB) and incubated for 18-24 h at 37 ^{0}C for enrichment. A loopful of broth culture was streaked on to Mannitol salt agar (MSA) and incubated for 18-24 h at 37 ^{0}C. The characteristic colonies were purified on Brain Heart Infusion agar. *Staphylococci* were tentatively identified based on colony morphology, pigment production, Gram's staining, catalase and oxidase tests as per the standard protocol. For the isolation of *E. coli*, all 236 samples were inoculated in MacConkey lactose broth (MLB) for enrichment and incubated for 18-24 h at 37 ^{0}C. A loopful of broth culture was streaked onto MacConkey lactose agar plates (MLA) and incubated at 37 °C for 18–24 h. Phenotypic identification of *E. coli* was carried out based on colony morphology, lactose fermentation, Gram's staining, IMViC tests (indole methyl red; Voges–Proskauer; citrate utilization) and oxidase test (Source of media: Himedia Laboratories, Mumbai, India).

For molecular confirmation, DNA was extracted from pure culture using QIAamp DNA Mini kit as per the manufacturer's protocol (Qiagen, Germany). The quantity and quality of DNA was evaluated by using a NanoDrop2000 (Thermo Scientific, Waltham, USA) and on 0.8% agarose gel electrophoresis respectively. Genus-specific PCR for the detection of *Staphylococci* (Shome et al. 2012) and *E. coli*-specific multiplex PCR (Modak et al. 2012) were performed. The ATCC 25922 and ATCC 33591 were used as positive controls for *E. coli* and *S. aureus* respectively. For identification of five major *Staphylococcus* spp. (*S. epidermidis*, *S. sciuri*, *S. chromogenes*, *S. haemolyticus*, and *S. aureus)* multiplex PCR was performed (Shome et al. 2011).

Antimicrobial Susceptibility Testing (AST)

AST was performed by Kirby–Bauer disk diffusion method (Bauer et al. 1996) against 10 antimicrobial agents including cefoxitin, a marker of methicillin resistance for *Staphylococcus* isolates. *Staphylococcus aureus* (ATCC-25923) was used as a quality control strain. Minimum inhibitory concentration (MIC) was determined only for vancomycin by broth microdilution method. The *E. coli* isolates were tested against 17 antibiotics representing different classes of antibiotics (penicillins and beta-lactams, cephems, carbapenams, aminoglycosides, tetracycline, quinolone, folate pathway inhibitors, phenicols and polymyxin). The results were entered in WHONET software (version 5.6) and were categorized as susceptible, intermediate, and resistant based on CLSI guidelines (2021). Isolates non-susceptible to at least one agent in ≥3 antimicrobial classes were categorized as Multidrug resistant (MDR) (Magiorakos et al.

2012). The ESBL and AmpC producing *E. coli* isolates were confirmed by combined disc assay method and for colistin assay, broth microdilution was performed.

Molecular detection of determinants of antibiotic resistance

The phenotypically resistant *Staphylococcus* isolates were characterized for the detection of methicillin resistant determinant *mec*A and *mec*C genes as previously described (Al Talib et al. 2009; Christiane et al. 2011). *E. coli* isolates resistant to third and fourth generation cephalosporins were investigated for the presence of CTX-M, SHV, TEM, and OXA. Further, cefoxitin and cefotetan resistant isolates were screened for AmpC, ACC, FOX, MOX, DHA, CIT and EBC genes. Additionally, the carbapenem resistant strains were screened for NDM, IMP, VIM and KPC (Dallenne et al. 2010; Koovapra et al. 2016).

Results

Staphylococcus spp: Identification, antibiogram and detection of methicillin resistance

Out of 128 milk samples (cattle- 105; buffalo-23) processed for *Staphylococcus* spp., a total of 113 (88%) *Staphylococcus* spp. isolates were identified by genus specific PCR. The highest number of *Staphylococcus* spp. were recovered from the milk samples of Gauribidanur cluster (n = 33) as shown in Table 1. Overall, 26% (n = 29) *S. aureus* and 49% (n = 56) Coagulase Negative *Staphylococci* (CNS) isolates were identified (*S. sciuri* = 23, *S. epidermidis* = 20 and *S. chromogenes* = 11 and *S. haemolyticus* = 2) (Fig. 2). In addition, 28 (25%) unidentified CNS isolates were also recovered (Table 2).

Table 2
Identification of different *Staphylococcus* spp. by species-specific multiplex PCR

Block	Village	*S. aureus*	*S. sciuri*	*S. epidermidis*	*S. chromogens*	*S. haemolyticus*	Total
Chikkaballapur	Kalavara	2	3	1	Nil	Nil	6
	Manchanabale	1	1	Nil	Nil	Nil	2
	Thumukalahalli	3	1	5	Nil	Nil	9
	Gundalagurki	Nil	3	2	Nil	Nil	5
	Subtotal	6	8	8	0	0	22
Gauribidanur	Haleupparahalli	2	Nil	1	5	Nil	7
	Hosuru	1	Nil	1	1	Nil	3
	Mudigere	Nil	3	Nil	1	Nil	4
	Kadalaveni	5	2	1	Nil	1	9
	Subtotal	8	5	2	7	1	23
Sidlaghatta	Kakachokkondahalli	Nil	Nil	4	Nil	1	5
	Nagamangala	4	1	1	Nil	Nil	5
	Gattamaranahalli	1	1	Nil	2	Nil	4
	Jangamakote	1	2	Nil	Nil	Nil	3
	Subtotal	6	3	5	2	1	17
Chintamani	Kendanahalli	Nil	Nil	4	1	Nil	5
	Thimmasandra	2	2	Nil	1	Nil	5
	Nayanahalli	4	1	Nil	Nil	Nil	5
	Konapalli	3	3	Nil	Nil	Nil	6
	Subtotal	9	6	4	2	0	21
Grand Total		29 (25.66%)	23 (20.35%)	20 (17.69%)	11 (9.73%)	2 (1.76%)	85 (75.22%)

All the staphylococcal isolates showed highest resistance to penicillin (70%) followed by cefoxitin (48%) whereas all the isolates were susceptible to Vancomycin. Other antibiotic tested showed resistance rate < 10% (Fig. 3). Methicillin resistance based on the cefoxitin disk diffusion test was detected in 54 of 113 *Staphylococcus* isolates (48%) and of which, 42 and 12 were from cow and buffalo milk samples, respectively (Table 3). On the contrary, PCR assay detected *mec*A gene in 13 (12%) isolates, and interestingly all of them were CNS [*S. sciuri* (n = 6), *S. epidermidis* (n = 4), *S. chromogenes* (n = 2) and *S. haemolyticus* (n = 1)]. Out of 13 *mec*A isolates, five isolates were sensitive to cefoxitin. Out of 54 cefoxitin resistant isolates, eight isolates alone are *mec*A positive (Table 4) and the rest forty-six isolates were *mec*A negative. None of the isolate harbored *mec*C gene (Fig. 2A)

Table 3
Antibiotic susceptibility pattern of *Staphylococcus* spp. isolates from cow and buffalo milk samples

Antibiotics (Concentration -µg)	Cow (n = 89)	Buffalo (n = 24)
Penicillin (10)	61 (68.5%)	18 (75%)
Cefoxitin (30)	42 (47%)	12 (50%)
Erythromycin (15)	8 (18.35%)	1 (4.16%)
Linezolid (30)	8 (18.35%)	1 (4.16%)
Enrofloxacin (5)	5 (5.62%)	1 (4.16%)
Trimethoprim-Sulfamethoxazole (1.25/23.75)	4 (4.49%)	3 (12.5%)
Tetracycline (30)	5 (5.62%)	1 (4.16%)
Gentamicin (10)	4 (4.49%)	0
Chloramphenicol (30)	3 (3.37%)	0

Table 4
The antibiotic susceptibility pattern of *mec*A positive 13 *Staphylococcus* isolates

Antibiotics	Resistance (n)	Zone of Inhibition (SE)	Intermediate (n)	Zone of Inhibition (SE)	Susceptible (n)	Zone of Inhibition (SE)
Penicillin	11	18.09 ± 2.85	0	0	2	35 ± 5
Cefoxitin	8	18.5 ± 1.43	0	0	5	26.6 ± 0.81
Erythromycin	2	8 ± 2	3	18.25 ± 2.17	8	26 ± 1.18
Linezolid	1	30	0	0	12	32.91 ± 1.47
Enrofloxacin	3	13 ± 1.88	2	17 ± 1	8	28.75 ± 1.13
Trimethoprim-sulfamethoxazole	3	7 ± 1.6	0	0	10	26.8 ± 1.98
Tetracycline	1	10	1	18	11	27.36 ± 1.48
Gentamicin	2	12	1	14	10	23.1 ± 1.59
Chloramphenicol	0	0	0	0	13	25.9 ± 1.19
Vancomycin	0	0	0	0	13	1.01 ± 0.08
*n represents number of isolates, SE represents Standard Error						

E. coli: **Identification, antibiogram and detection of antibiotic resistant genes**

Out of 236 samples, a total of 112 (47%) *E. coli* isolates were identified by multiplex PCR assay (Fig. 4) and highest number of *E. coli* were recovered from Chikkaballapur cluster (n = 36), followed by Sidlaghatta (n = 30) (Table 1). The *E. coli* from various sources [Poultry-89% (40/45), sheep – 89% (41/46), goats-73% (11/15), pigs-100% (2/2), cow milk-15% (16/105) and buffalo milk-8.7% (2/23)] were isolated. Majority of the *E. coli* isolates were from cloacal swab samples from poultry and sheep.

Antibiotic susceptibility profiles of *E. coli* isolates showed complete resistance against cefotaxime (29%), followed by ampicillin and nalidixic acid (both 23%). The other cephalosporins showed resistance rate in the range 8–16%. None of the *E. coli* isolate appeared completely or intermediately resistant to colistin and 50% of the isolates showed intermediate resistance against cefotaxime followed by ceftazidime (25%). Overall antibiotic profile of *E. coli* isolates is depicted in Fig. 5.

Based on the double disk synergy test, 6% (n = 7) and 4% (n = 5) of *E. coli* isolates were identified as ESBL and AmpC producers, respectively and 3% (n = 3) were of both ESBL and AmpC producers. Overall prevalence of MDR in *Staphylococcus* spp. was found 20.35% and 35.71% in *E. coli* (Fig. 6). Maximum MDR prevalence (12%) was recorded in Chikkaballapur cluster. Out of 15 phenotypically identified ESBL and AmpC producers, 10 isolates were confirmed for the presence of resistance genes: 7 isolates of ESBL producers [poultry origin (n = 4), cow (n = 2) and sheep (n = 1)], one *Amp*C producing *E. coli* from cow, one isolate from poultry carried both ESBL and *Amp*C producers and one isolate from pig carried both *Amp*C and NDM genes.

Discussion

In this cross-sectional study, the prevalence of multidrug resistant *Staphylococci* and *E. coli* was assessed from livestock milk samples. As per several studies, *Staphylococcus* are the most isolated bacteria from milk of dairy cows (Mork et al. 2012) and similar observation was also documented in the present study with 88% recovery rate of *Staphylococci*. There are two types of *Staphylococci*, CNS is often considered as opportunistic pathogens isolated from extra

mammary sites such as bovine skin and teats (Taponen et al. 2009) and coagulase-positive staphylococci (*S. aureus*) is considered as a major pathogen whose primary mode of transmission is from one animal to other. Current study has detected majority of staphylococcal isolates as CNS (74%) with only 26% as *S. aureus*. In another study from the same region, CNS prevalence was 76% of which 42% was identified as *S. epidermidis* in livestock (Venugopal et al. 2019). In this study, the most predominant CNS species was *S. sciuri* (20%), followed by *S. epidermidis* (17.7%) from milk samples. A report from Brazil recorded *S. warneri* (32%) as most predominant species in bovine mastitis (Guimaraes et al. 2016).

Highest resistance rate against penicillin (70%), followed by cefoxitin (48%) was observed in staphylococcal strains in our study, whereas mastitis milk samples from Ludhiana, Punjab state reported highest resistance to amoxicillin (77%) followed by penicillin (75%) (Bansal et al. 2015). The CNS isolates from hospital setup in Jaipur showed high resistance to penicillin G followed by amoxyclav (Kumar et al. 2018). Similar findings from sub clinical mastitis milk samples from Karnataka showed high resistance to methicillin, penicillin and amoxicillin in the range of 72 to 86% (Preethirani et al. 2015).

The *mec*A gene is highly conserved in staphylococcal strains and thus it is used as a useful marker and considered as gold standard for methicillin/oxacillin resistance detection of methicillin resistant Staphylococci (Ferreira et al. 2003). The present study detected 12% of *Staphylococcus* spp. harboring *mec*A gene by PCR and all the *mec*A positive isolates were of CNS. Methicillin resistant CNS from livestock sector was lower (6.8% prevalence) in other study from India (Venugopal et al. 2019). In hospital setups, presence of *mec*A gene was very high in both *S. aureus* (75%) and CNS isolates (64%) (Jain et al. 2008). Several researchers have reported that the results of cefoxitin disk diffusion method correlates better for the presence of *mec*A as compared to oxacillin disk diffusion method (Swenson et al. 2005; Felten et al. 2002). We observed the phenotypic cefoxitin disc diffusion showed higher 48% resistance than genotypic mecA gene detection (12%) in Staphylococci isolate.

In this study, recovery rate of *E. coli* in milk samples was low (in the range of 8–15%) compared to cloacal or rectal swabs (in the range of 70–100%). Always higher prevalence of *E. coli* was reported from poultry cloacal samples (78.86%), in ready-to-eat milk products (76%), followed by ready to eat meat products (35.21%) from Assam (Barua et al. 2007). In neighboring country Bangladesh, 37.86% prevalence of *E. coli* was recorded in foods of animal origin (Rahman et al. 2017). Such variations in prevalence might be due to the variation in the selection of sample source, processing and the antibiotic load in the sample in different geographical areas.

The resistance against the two important antibiotics fluroquinolones and cephalosporins, a third and fourth generation, respectively in *E. coli* is increasing worldwide in veterinary and human medicine. Resistance of *E. coli* strains to multiple antimicrobials could result in failure of antibiotic therapy and can pose a serious threat to human and animal health. In the present study, complete resistance against cefotaxime (29%), followed by ampicillin and nalidixic acid (both 23%) was observed. However about 50% of the isolates are in intermediate range, which implies an alarming situation as the isolates are on the verge of obtaining resistance. From clinical samples of livestock, *E. coli* was recovered at 62% with 17% prevalence of ESBL by using confirmatory disc diffusion method (Sharif et al. 2017). Higher rate of 77–93% was observed for penicillin, neomycin and streptomycin with 67% of the isolates MDR from diseased food animals from Malaysia (Haulisah et al. 2021) and one health study from Canada confirmed ESBL phenotypes in 24.7% of E. coli isolates (Adator et al. 2020).

Antimicrobials have been widely used in animal husbandry to prevent disease, reduce infections and promote animal growth. The European Union no longer allows usage of antimicrobial as growth promoters, but is still currently practiced in India and many other parts of the world (Bonnet et al. 2009). Antimicrobial usage has resulted in selection for antimicrobial-resistant *E. coli* in the commensal microbiota that eventually contaminate humans as they consume contaminated meat (Aarestrup et al. 2001). Studies have reported a high rate of antimicrobial MDR *E. coli* in broiler chickens (Diarra et al. 2007; Heuer et al. 2002) and 78% of MDR prevalence in India (Hussian et al. 2019). Different resistance patterns were observed to several old antibiotics that have been commonly used in treatment of animals for a long time (Gong et al. 2013) and these are mainly tetracycline, nalidixic acid, and ampicillin. Although the present study showed low resistance to tetracycline (20%) which is contrast to 75% resistance observed in poultry and livestock samples (Adator et al. 2020).

The current study showed 35.71% MDR prevalence in various food animals whereas 66.20% of MDR from bovine clinical samples was reported from the same region (Armanullah et al. 2018). Similarly, as high as up to 50% MDR prevalence reported from Bangladesh in broiler chicken (Rahman et al. 2020). The prevalence of MDR was comparatively low, because of the surveillance samples collected from unorganized farming as many reported works are based on clinical samples the prevalence rate was high.

As per our study in foods of animal origin showed 13% of ESBL and AmpC producers by confirmatory double disc synergy test, whereas 9% of the *E. coli* isolates carried ESBL resistant marker genes and AmpC beta lactamase genes. High prevalence of ESBL producers (40.07%) and AmpC producers (14.8%) were detected in tertiary care centers in India (Grover et al. 2013). In United States, prevalence of ESBL producing isolates was reported to be 75% from 24 medical centers (Moland et al. 2002). The results are in concordant with a study reported from Odisha from poultry and cattle milk (Kar et al. 2015). In tertiary healthcare setting, NDM gene was reported more commonly because of their dissemination (Bora et al. 2013; Rahman et al. 2014; Ahmad et al. 2018), however from India, the NDM was reported from mastitis milk sample (Ghatak et al. 2013) and pigs (Pruthvishree et al. 2017) from North Eastern states. This is the first report as per author's knowledge for the identification of NDM gene positive isolate from livestock in unorganized farming sector from southern state which raises concern for the likely transmission of resistance genes from animal to human.

Comparative study of sample collection across the animal species from 4 different clusters showed variable pattern of resistance, Chikkaballapur cluster showed highest level of resistance followed by Sidlaghatta and Gauribidanur clusters, which showed moderate level of resistance whereas, Chintamani cluster showed least level of resistance determinants. Interestingly, methicillin resistant staphylococci could not be detected in Chintamani block.

Conclusions

The present study reports increasing rate of methicillin resistant *Staphylococcus spp.* and β-lactam resistant *E. coli* circulating within the livestock across the study sites. The occurrence of multiple resistant determinants and NDM gene in these isolates relate to complex antimicrobial resistance. The different

dynamics of circulating AMR genes in our study represents an alarming concern for both animal health as well as in contact human subjects. They can act as vectors to spread antibiotic resistance at a high rate. Therefore, it is necessary to be cautious to decrease the incidence of multidrug resistant strains of *Staphylococcus* and *E. coli* in animals and humans. In order to achieve this, good hygienic practices are necessary from the farm to the family table especially in the abattoirs to prevent contamination of cattle and poultry products and abattoir environment with intestinal content. Strict law enforcements need to be followed by health authorities on usage of untreated sewage water or animal waste for vegetable growing. To protect the available antimicrobials, the concept of reserve drugs should be strictly followed to avoid irrational use. In addition, regular strategically planned antimicrobial susceptibility surveillance is essential. Thus, the study advocates the necessity for continuous monitoring of resistance trends at genomic level and enhancing the infection control of these pathogens in animal settings.

Declarations

Code availability

Not applicable

Funding

No funding.

Data availability

Data used to support the findings of this study are available from the corresponding author upon request.

Author's contribution

Conceptualization: Shome B R; Data curation: Ojha R, **Mendem S K**; Formal analysis: Murugesan D; Funding acquisition: Shome B R; Sample collection and processing: Sridevi R, Lekshmi JD, Tewari R, Murugesan D, Venugopal N; Project administration: Shome R, Shome B R; Resources: Shome B R; Supervision: Shome R, Shome B R; Visualization: Murugesan D, Chanda M, Ojha R, **Mendem S K**; Writing - original draft: Murugesan D, Tewari R; Writing - review & editing: Shome R, Sivasharanappa N, Shome B R.

Ethics approval

This study was approved by the ICAR-NIVEDI Ethics Committee. No human or animal experiments were conducted during this study.

Consent to participate

Animal handlers' consent was taken for participating in the study and for sample collection.

Consent for publication

All author's consent was taken for publication

Conflict of Interest

The authors certify that there is no conflict of interest.

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Figures

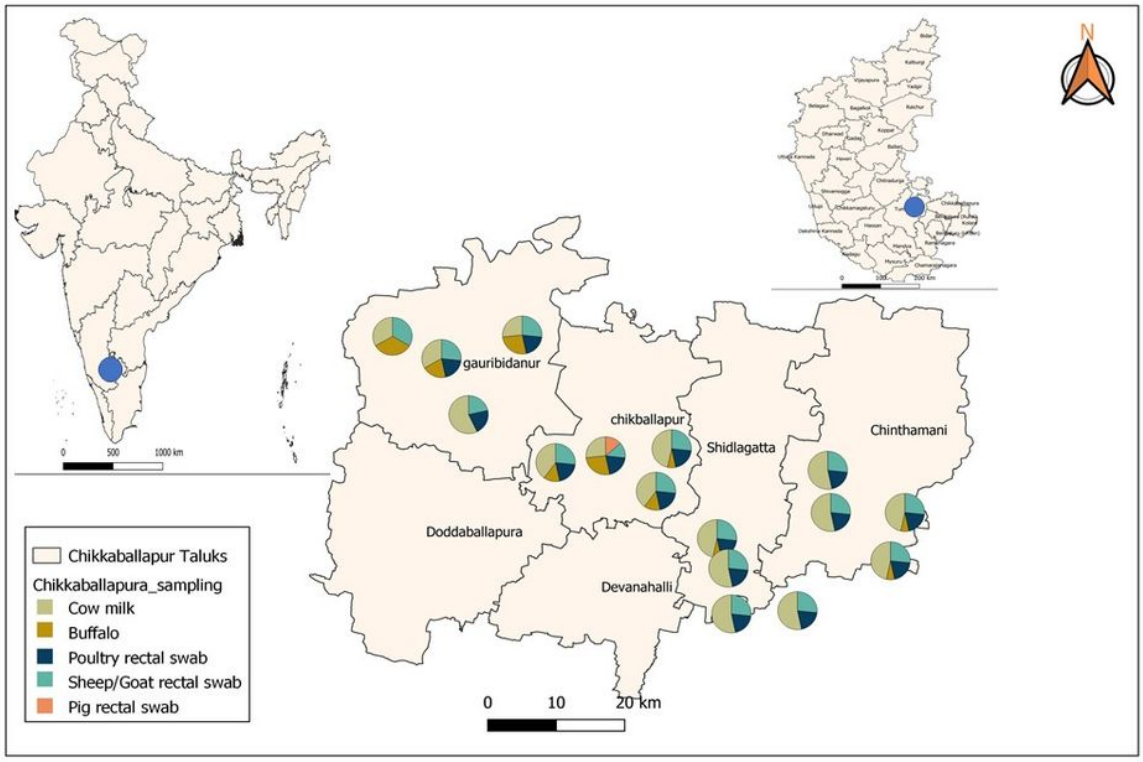

Figure 1

Map showing the sample collection sites with Staphylococcus and *E. coli* distribution in Chikkaballapur district, Karnataka, India

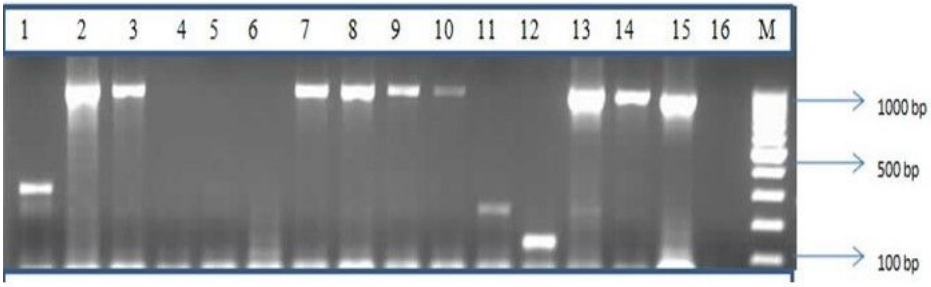

Figure 2

Multiplex PCR- Species level identification of Staph species. mPCR for *Staphylococcus* species- Lane 1: *S. sciuri*(306bp), Lane 2, 3, 7-10, 13, 14: *S. aureus* (894bp), Lane 11: *S. chromogenes*(222bp), Lane 12: *S. epidermidis* (130bp), Lane 15: ATCC 25904- positive control for *S. aureus*, Lane 16: NTC, Lane 17: 100bp ladder

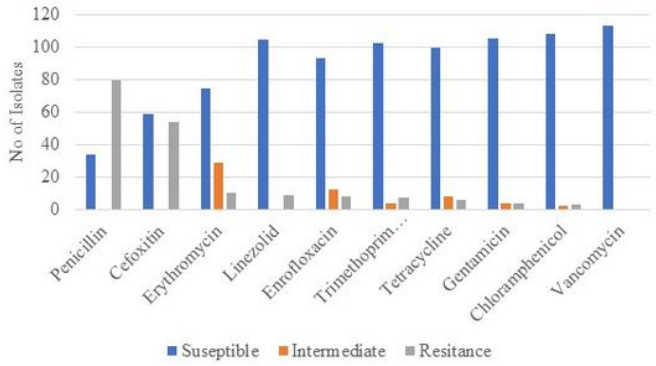

Figure 3

Antibiotic susceptibility profile of *Staphylococcus* isolates

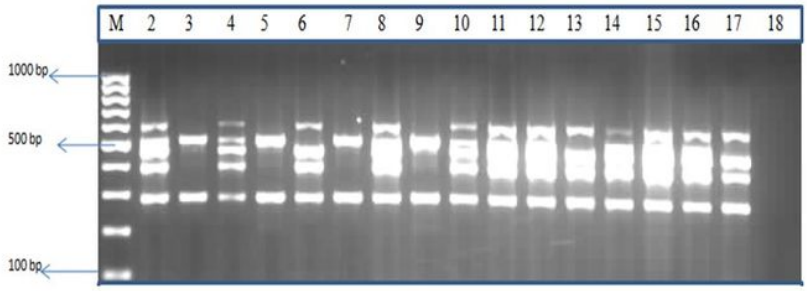

Figure 4

Multiplex PCR for Identification of *E. coli.* Lane 1: 100bp ladder, Lane 2,4,6,8,10-16: Field isolates positive for *E. coli*, Lane 3, 5, 7, 9: negative for *E. coli*, Lane 17: ATCC 25922- positive control for *E. coli*, Lane 18: NTC

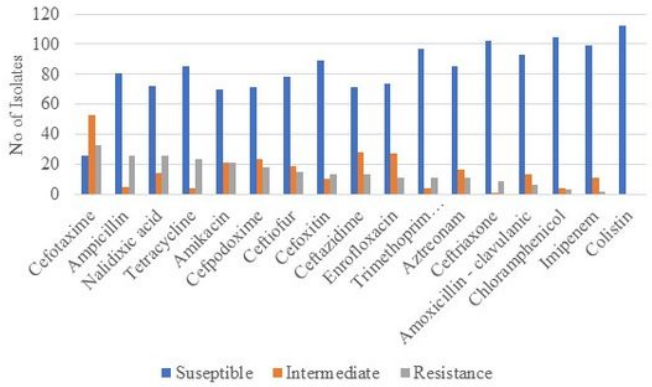

Figure 5

Antibiotic susceptibility profile of *E. coli* isolates

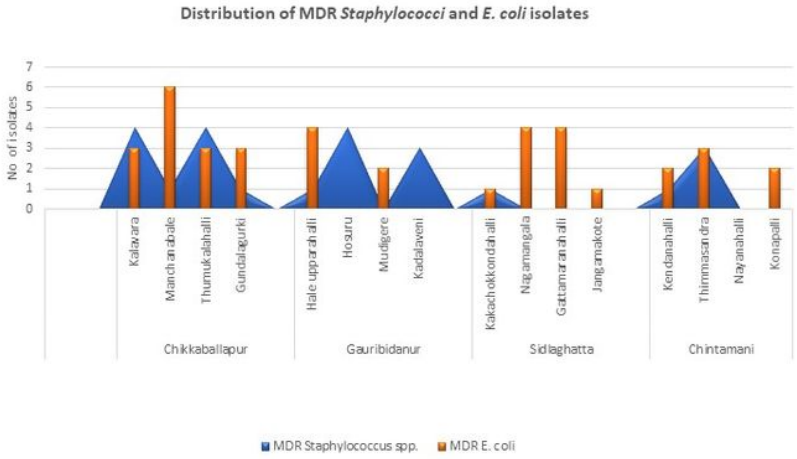

Figure 6

Multiple drug resistance pattern of *Staphylococcus* and *E. coli* isolates

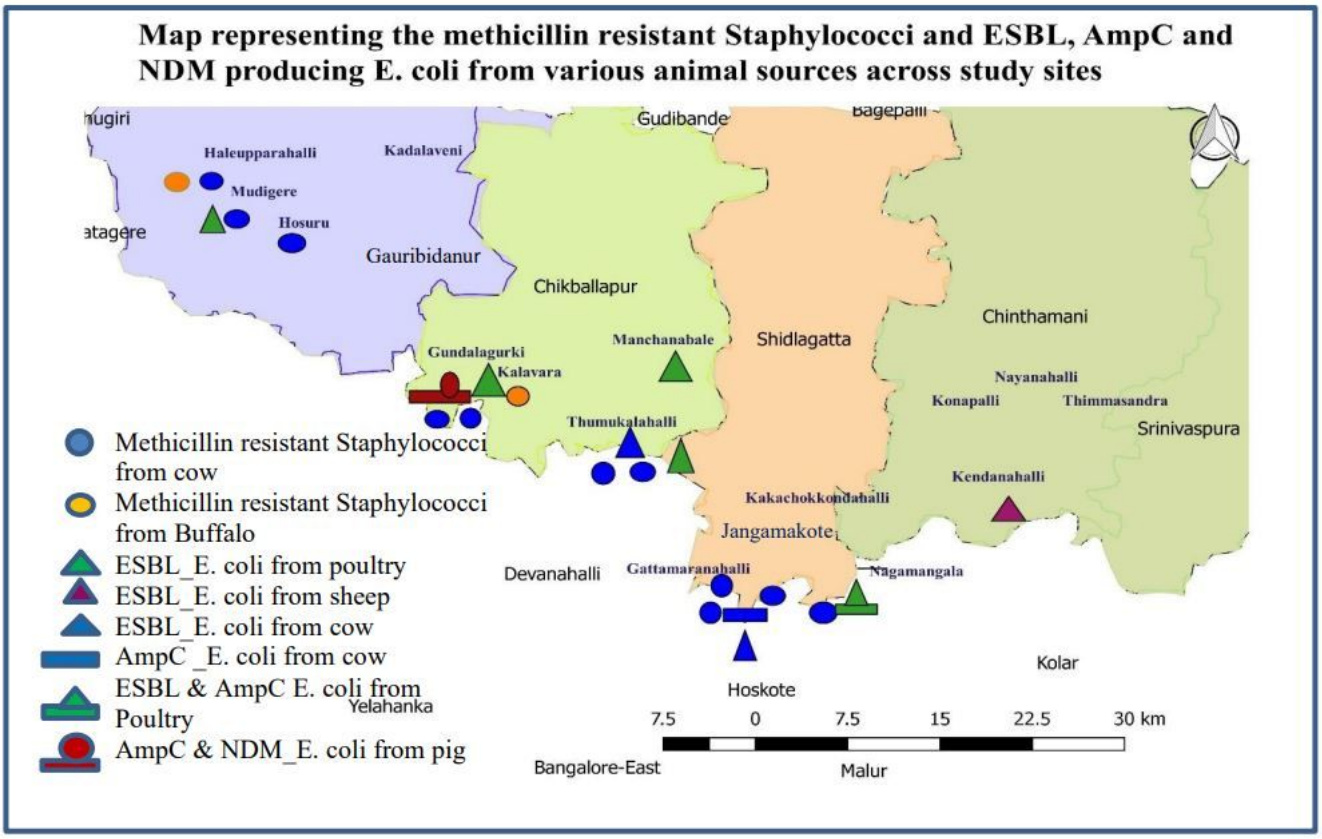

Figure 7

Represents methicillin resistant Staphylococci and ESBL, AmpC and NDM producing *E. coli* from various animal sources across four study sites. Blue color indicates cow, yellow indicates Bufallo, green indicates poultry, red indicates pig and pink indicates sheep.