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Determination of Carbapenem and Extended Spectrum Beta Lactamases in *E. Coli* from Commercial Broilers, Sri Lanka

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Authors' contributions

This work was carried out in collaboration among all authors. Author MARP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PSDA and PSF supported laboratory work and managed the analyses of the study. Author MARP and PSF managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Association between antimicrobial resistance in human and livestock have been widely discussed. The same or closely associated sequence type of *E. coli* has been reported in poultry and causing clinical infection in humans. The objective of this study was to determine phenotypic and genotypic antimicrobial resistance among *E coli* isolated from commercial broiler integrators (n=6) in the country. Ceacal samples (n=521) were collected from commercial broiler processing plants and *E.coli* was isolated and identified by conventional bacteriological methods followed by selected biochemical tests. Antimicrobial susceptibility testing and interpretation were done by disk diffusion tests as described in EUCAST. The conventional PCR tests were carried out for CTX-M for ESBL phenotypically resistant isolates and NDM for meropenem resistance isolates. NDM and CTX-M were found in 0.38% and 18.2 % in *E. coli* respectively. High frequency of phenotypic resistance was observed against neomycin (100%), tetracycline (99%), ampicillin/amoxycillin (98%), and quinolone (91.5%), gentamicin (79%), and over 50% of the frequency of antimicrobial resistance

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were shown against amoxycillin + Clavulanic acid, streptomycin and chloramphenicol. All these classes of antimicrobial are widely used in commercial broiler operations in the country. NDM has not been reported in *E. coli* from commercial poultry previously, although NDM had been reported in human clinical isolates. Prudent usage of antimicrobials, strengthening resistance surveillance, molecular epidemiological studies, and understanding the role of the mobile genetic elements are strongly recommended to minimize the risk of dissemination of antimicrobial resistance in humans.

Keywords: E. coli; AMR; poultry; Sri Lanka.

1. INTRODUCTION

Antimicrobial resistance still remains as an emerging threat in human and veterinary medicine [1]. In addition, antimicrobial resistance has been identified as a significant cause of loss on livestock production and ultimate effect in the economy of a country [2]. Specially developing countries are facing huge challenges to keep away antimicrobials in livestock production systems [2]. Triggered pressure on usage by inappropriate usage of antimicrobials, unauthorized sales and extensive usage have been identified as major factors on the emergence of antimicrobial resistance in livestock production [2]. However, carbapenem, the third-generation cephalosporin and colistin have been listed as the highest priority drug in human medicine by WHO and those drugs are not recommended on using livestock production [2]. Moreover, carbapenem is the last-line drug for treating Gram-negative infection in humans [3-4] In addition, it has been reported that some members of the family *Enterobacteriaceae* are survived with a clinically relevant concentration of carbapenem [4].

Sri Lanka is considered as one of the endemic countries for NDM in South Asia together with India and Bangladesh [5-6]. NDM, SHV has been reported among human patients in the region [7] [6]. Although the exact source of carbapenem resistance is not known, the association of antimicrobial resistance in livestock and human have been investigated extensively [8-9]. Furthermore, livestock is considered an essential source of protein in human food production while the chicken is contributed as a major portion of animal protein in the World [10]. Although usages as growth promoters have been banned, antimicrobials are widely used for prophylactic and therapeutic purpose in the industry [9].

E. coli is being considered as the reservoir of many antimicrobial resistant genes in which could be transferred to pathogen found in human [2]. Association of *E. coli* between human and poultry has been suggested by many studies outside the country, the significant epidemiological association between carbapenem resistant *E. coli* has not been detected in commercial poultry in Sri Lanka [11]. In addition, extended spectrum beta lactamases (ESBLs), such as CTX-M have been reported common in *E. coli* from commercial poultry [12]. The CTX-M- 15 was reported common both in poultry and human [12]. Therefore, the objective of the study was to explore the NDM resistance in *E coli* from poultry integrators in the country.

2. MATERIALS AND METHODS

The samples were collected from 6 big poultry integrators in the country. The caecum of slaughtered broilers was collected from different flocks submitted for slaughtering on that day and caecum were cultured on MacConkey agar and incubated at 37^0C overnight. The Next day only pink colonies were selected and a maximum of three isolated colonies from each plate was selected randomly, sub-cultured and incubate overnight. The isolated cultures were done series of biochemical tests to identify the organism as *E. coli* [13]. Only identified *E. coli* were sub-cultured into a new MacConkey plates and incubate overnight at 37^0C [14]. The following day, the isolates which grown were TSB with 15% glycerol and stored in -40^0C for further testing in the laboratory [13]. Only single *E. coli* isolate per bird was selected for antimicrobial susceptibility testing. Altogether 521 (n=521) number of *E. coli* isolates were collected from commercial poultry at the slaughterhouse of poultry integrators in the country.

Each isolate was stored in were sub-cultured in 5% sheep blood agar and incubated at 37^0C overnight. Antimicrobial susceptibility testing was done by disk diffusion test as described by EUCAST for different classes of antimicrobials such as beta lactams, aminoglycoside, chloramphenicol, quinolone, tetracycline,

sulfamethoxazole, and trimethoprim (www.eucast.com). The DNA extraction was made by conventional of boiling methods (95^0C for 10 minutes and centrifuge 5000 rpm for 5 minutes and supernatant wet collected as DNA mix) [13]. The conventional PCR for CTX-M was done as described by Pitout et al. [15] while PCR for NDM was done as the method described by Gayer & Hensen, 2013 on phenotypically resistant isolates [16] [15].

3. RESULTS

The high percentage of antimicrobial resistance was observed by *E. coli* for neomycin, tetracycline, beta lactams such as ampicillin/amoxicillin, tetracycline, and quinolones Table 1. The extracellular beta lactamase production was high as 64% in the study. In addition, more than 50% of resistance was reported for gentamicin, streptomycin, and chloramphenicol. The phenotypic resistance was observed low only for extended spectrum beta lactamases and carbapenem only. In addition, 0.38% and 18.2% of isolates were shown positive for NDM and CTX-M respectively Table 2.

Table 1. Percentage of phenotypic antimicrobial resistance in *E. coli* isolated from commercial broilers

Antimicrobial agent	Resistance %
ampicillin	98.2
amoxicillin	98.3
neomycin	100
Amoxicillin-Clavulanic acid	64.4
streptomycin	64.4
gentamicin	79.0
ciprofloxacin	91.5
chloramphenicol	51.1
Sulfa trimethoprim	31.4
tetracycline	99.0
Cefotaxime	24.2
meropenem	14.1

Table 2*. E. coli* isolates those were positive for CTXM and NDM by conventional PCR test from commercial broilers

Resistant gene	Percentage
CTX-M	18.2% (n= 101)
NDM	0.38 % (n= 2)

4. DISCUSSION

E. coli is commensal bacterium in gastro intestinal tract of human and livestock [2]. Furthermore, *E. coli* is considered as an indicator organism on antimicrobial resistance due to the presence of inherited characteristics such as widespread, an indication of selective pressure in all relevant microbial population and reservoir of multiple resistant genes [17]. As mentioned previously, *E. coli* is part of the microbial flora of the gastrointestinal tract (GIT) of poultry and clinical infection may cause under certain conditions in chicken [18-19]. [13] [9]. Importantly, the majority of antimicrobial resistant genes are found in mobile genetic elements and dissemination of resistance is not a challenging task within a localized microbial population [20] [17].

Bacterial infections are common in livestock farming and usage of antimicrobials is not an unusual practice in animal husbandry including chicken farming [21]. However, antimicrobials are used only for therapeutic purposes in poultry since antimicrobial growth promoters had been banded in Sri Lanka by recent governmental decision taken by Department of Animal Production and Health (DAPH). However, malpractices and over usage of antimicrobials are still found in the industry although exact scientific evaluation on antimicrobial usage has not been done in veterinary practices in the country recently. In the global context, antimicrobial resistance in commercial poultry is a challenging issue due to emerging multi drug resistance in a different microorganism [22-23]. Multidrug resistance (MDR) has been reported in many bacterial pathogens and commensals such as *E coli* [4]. Therefore, *E coli* is considered as an indicator organism for antimicrobial resistance while the same pathogenic strains are shared by both humans and poultry [22]. The frequency of resistance to each antimicrobial by *E. coli* may change due to a number of factors such as management practices, type of feed, hygienic condition, geographic location and other unknown factors [24]. Therefore, the frequency of antimicrobial resistance may be varied in Europe, North America, and Asia and South America. The only practical alternative to know the emerging resistance in microbes is the resistant surveillance representing all possible stages in the production.

Carbapenem resistant gene *bla* $_{NDM}$ has not been reported in *E. coli* from commercial poultry previously [25]. Importantly, NDM is found in a small mobile genetic element such as plasmids and antimicrobial resistance might be transmitted from poultry to humans by working in livestock

farm premises, consumption or handling of infected or contaminated food [25]. NDM carrying mobile genetic elements or plasmids were reported both in commercial broiler and layers [25]. *E. coli* ST 156 have been recognized as one of the NDM carrying sequence types in China [25]. Sri Lanka has been identified as high colonization of NDM in the country since travelers were confirmed positive for NDM infection [18] [26]. However, limited studies are found in the field of livestock.

The prevalence of ESBL resistance in poultry has been increased last two to three decades in the world [27]. In addition, ESBL resistance has been identified as a main threat in human and livestock [23]. In ESBL resistance, CTX-M were identified as common category found in poultry while CTX- M- 15 was found common in poultry and human [23]. CTX -M- 1 was dominant in *E. coli* isolates from clinical diseases both in human and livestock [27]. In contrast to carbapenem resistance, ESBL resistant gene are found in large plasmids, dissemination of resistance may be difficult than in small mobile genetic elements [27]. *E. coli* ST 131 were the common sequence type found in human specially in hospital acquired infections [19] [27]. In addition, CTX-M-1, TEM-52 and SHV-12 were identified as the most common ESBL genes found in commercial poultry all around the World [23].

On the other hand, limited literature is found on ESBL and carbapenem resistance in *E. coli* from human clinical submission in the country. According to Thilakerathne et al, 90% of *E. coli* reported from Urinary tract infection in humans carried CTX-M gene while 40% of UTI infection were reported on ESBL resistance [5]. Similarly, high frequency of resistance was reported in *E. coli* from a patient with blood born infection in Sri Lanka [28]. In addition, resistance to meropenem was observed high as 95% in *E. coli* from human UTI in Sri Lanka [29]. However, the exact source of these clinical infections has not been identified.

A high frequency of resistance was reported on beta lactams by *E. coli* from commercial poultry in other countries [1-2]. Resistance to ampicillin and amoxicillin were high as 82% and 80% in *E. coli* from commercial poultry, respectively [1]. In addition, resistance to beta lactam and chloramphenicol were 69% and 52% in Brazil and 81% and 41% in China [1] [17]. The high frequency of resistance to gentamicin (31%), chloramphenicol (63.5%), ciprofloxacin (67%), tetracycline (91%) and sulfa trimethoprim (60.8%) was reported [22]. The CTX-M was identified as the most common resistant gene in extended spectrum beta lactamases in *E. coli* from commercial poultry and it was 59% [15] [17] [23]. Emerging multi drug resistance has been identified as a critical issue in Gram negative organisms from clinical infections [30]. Importantly in this study, NDM was isolated less than 0.5% genotypically although phenotypic resistance to carbapenem were observed high as 17.9%. A high percentage of New Delhi metallo-β-lactamase (NDM) has been detected in commercial poultry operations previously as 18% in day-old chicks in a Chinese poultry farm [31] As mentioned previously, resistance surveillance is the only method to identify the emerging risk of resistance in livestock [31]. Further short sequencing of the NDM gene is strongly recommended although that has not been done in the study due to financial limitation and shortage of resources locally. However, an extensive study on determination and further characterization of NDM and CTX-M are highly recommended for future studies. In addition, molecular epidemiological-based-antimicrobial resistant surveillance is limited in the country.

In this study, resistance to beta-lactam, aminoglycoside, quinolone, tetracycline was shown high. Resistance to chloramphenicol was over 50% and over 30% of isolates were observed resistant to sulfa trimethoprim. Chloramphenicol, streptomycin is not used currently in the industry and livestock preparation is also not found. However, beta lactams, sulfa-trimethoprim, neomycin, and other aminoglycosides are widely used in the poultry industry. Specifically, neomycin is used widely against Gram negative infection in poultry. Furthermore, the quinolone class of enrofloxacin and tetracycline is still used widely in local poultry. These two classes of antimicrobials are commonly used in the first two weeks of commercial broiler farming for *E. coli* and *Salmonella* infections in chickens. In contrast, a low frequency of resistance had been reported for tetracycline, quinolone and aminoglycoside in *E. coli* from poultry in neighboring country India [32]. Importantly, a significant amount of antimicrobials such as tetracycline (0.004-0.005 ppm) and beta lactam were found in the waste of poultry farming in Sri Lanka [21]. oxytetracycline were found common as 55% of farm examined in the study [21]. In addition, high concentration of tetracycline and enrofloxacin were found in poultry litter where birds are reared in the same

study [21]. Therefore, exposure for these antimicrobials and antimicrobial residues may be one of the reasons for high frequency of antimicrobial resistance in the study [33]. Furthermore, housing system and frequency of antimicrobial resistance has been proven association with high frequency of antimicrobial resistance in poultry [27]. Whereas highest resistance to antimicrobial were reported in conventional cages than barn system and free raging birds [34]. Multi drug resistance were 28% in the study and high multi drug resistance in *E. coli* from commercial poultry were reported as 62% , 83% in Canada and China respectively [34] [9]. However, similar finding such as high frequency of resistance were reported for tetracycline, quinolone and chloramphenicol were reported in chicken, Saudi Arabia [1].

Antimicrobial resistance surveillance is the only way to get information on going antimicrobial resistance in microbial populations associated with livestock. Resistance surveillance is an investment and all policies and strategies on antimicrobial usage in the future can be taken based on these findings. Although we have documented on strategies of antimicrobial resistance in the country, national level resistance surveillance is not carried out in the current livestock industry. Therefore, implementation of national resistance surveillance is strongly encouraged and identified as the main priority on controlling antimicrobial resistance in the country. In addition, understating mobile genetic elements with resistance genes are recommended with extensive resistance surveillance in the country.

5. CONCLUSION

The study concluded emerging antimicrobial resistance in *E. coli* for antimicrobials such as quinolone, tetracycline, beta lactam, aminoglycoside, and sulfamethoxazole in poultry. Although no high frequency of resistance was observed for ESBL and carbapenem in the study, importance of continue resistance surveillance were concluded. Multi drug resistance is also emerging in E. coli from commercial broilers as an indicator organism for antimicrobial resistance. Further extensive and national resistance surveillance for antimicrobial are strong recommended preventing further threat in antimicrobial resistance in livestock and human in the country.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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