Engineering and Scientific International Journal (ESIJ)
Volume 8, Issue 1, January – March 2021
ISSN 2394-7187(Online)
ISSN 2394 - 7179 (Print)

Antimicrobial Resistance: A Menace to Food Chain

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Abstract— Food animals including poultry are the important reservoirs of human enteric pathogens. Moreover, it is observed that many human infections are associated with consumption of food products of animal origin. In fact, there is a chance of indirect transmission of commensal and opportunistic bacteria of *Enterobacteriaceae* group present in poultry and pig gut to human through the food chain. Antimicrobials are often used in food animals for their treatment and prevention of diseases besides they are used as a growth promoter. The commensal bacteria, present in the livestocks are challenged by antimicrobial agents; thereby develop their survival strategies through mutations and adaptations. Thus antimicrobial resistance (AMR) emerges from the use of antimicrobials in animals that subsequently causes transfer of resistance genes and bacteria among animals/animal products entering in the food web. Potential routes of entry of bacteria having AMR property in different animal rearing systems, viz. broiler, kuroiler and indigenous poultry, duck, pig, goat, buffalo and cattle have been investigated at local levels in West Bengal, an eastern state of India. Evidence strongly suggests that besides conventional source of antibiotics for therapeutic intervention, use of antibiotic growth promoter (AGP) at sub-therapeutic doses might be an additional source of generation of AMR in backyard system, a menace to food chain.

***Keywords** — Antimicrobial Resistance (AMR); Food Animals; Food Chain; Growth Promoter.*

1. Introduction

Antibiotic resistance, considered to be an inherent bacterial trait, enables bacteria to survive and continual growth instead of being exposed to different anti-microbial agents at therapeutic doses. In fact, antibiotic-resistant bacteria evade the effects of antibiotics and multiply in their own way leading to severe health consequences in human and animals. The fast growing problem of antimicrobial resistance (AMR) is hindering treatment modalities targeted for infectious diseases. We frequently face hazards of antimicrobial resistance in various pathogenic and non-pathogenic organisms, viz. *Salmonella* spp., *Campylobacter* spp., *Yersinia* sp., *E. coli* serotype O157:H7, enterococci, *Pasteurella* sp., *Actinobacillus* sp. with varying severity [1].

The antimicrobial resistance is broadly of two types, viz. Intrinsic and acquired. The intrinsic type of resistance is seen in bacteria having no cell wall. On the other hand, acquired type is related to blocking of cell wall synthesis in normal bacteria. Moreover, it is a typical property encoded in the chromosome and shared by the member species. Bacteria can acquire resistance either by mutations in the genomic DNA or by getting exogenous DNA from a bacterium that is resistant through mobile genetic elements, e.g. plasmid, bacteriophage, transposons etc. Resistant genes are found to be situated on chromosome, plasmids, integrons or transposons [2].

Bacteria confers resistance to different antimicrobial agents by the following ways- i. By obtaining genes that encode enzymes (e.g. β lactamases) responsible for targeting antibacterial agent, ii. By having efflux pumps that remove the antibacterial agent out of the cell prior to its action, iii. By getting several genes meant for metabolic pathways responsible for production of dummy cell wall on which the antibacterial agent may be attached and iv. By inhibiting the porin genes is lowering the entry of antibacterial agents.

2. Curse of Antibiotic Resistance

The steep rise of antibiotic resistance among prevalent pathogens is a real hurdle to the management of infectious diseases. Besides human, various livestock animals, viz. domestic poultry, pig, beef cattle possesses antibiotic-resistant *E. coli* and *Salmonella*. In veterinary medicine, tetracycline is the most commonly used antibiotic. Other antibiotics used are of the groups of beta-lactams, sulphonamides, macrolides and aminoglycosides. Multidrug resistant *E. coli* are common in poultry, pig and cattle. In pig, Tetracycline resistant genes tetA and tetB were identified in clinical samples of pig, wheras, in pig, tetB and tetC genes representing tetracycline resistance were observed in normal gut microbiota. Beta-lactams being one of the important groups of antimicrobial agents, production of β-lactamase is the cardinal mechanism of resistance to β-lactam antibiotics in Gram-negative organisms [3]. Some of them produce chromosomal β-lactamase, whether constitutively or inducibly, e.g., *Klebsiella pneumoniae* produces class A β-lactamase constitutively, whereas, *Enterobacter aerogenes*, *Pseudomonas aerugenosa* produce inducible class C β-lactamase. On the other hand, plasmid mediated β-lactamases have become prevalent among Gram-negative bacteria, e.g. TEM-1, SHV-1 etc. [4]. The TEM-1 β-lactamase has spread worldwide and found in different members of *Enterobacteriaceae* group, *Pseudomonas* sp.,

DOI: **10.30726/esij/v8.i1.2021.81005**

ISSN 2394-7187(Online)
ISSN 2394 - 7179 (Print)

Haemophilus sp. etc. The SHV-1 β-lactamase was found in *Klebsiella pneumoniae* and *E. coli*. The extended spectrum β-lactamases (ESBL) are a class of lactamases that hydrolyze a broader spectrum of β-lactam antibiotics than the simple parent β-lactamases [5]. They have the ability to inactivate β-lactam antibiotics containing an oxyimino-group such as oxyimino-cephalosporins, viz. ceftazidime, ceftriazone, cefotaxime besides oxyimino-monobactam, viz. aztreonam. However, certain antibiotics are not challenged, viz. cephamycins and carbapenems. These are inhibited by lactamase inhibitors, viz. Clavulanate and tazobactam [6]. Moreover, AmpC β-lactamase-producing organisms produce resistance against cephalosporins, penicillins, cephamycins, monobactams, even against β-lactamase inhibitors such as clavulanic acid [7].

It is assumed that loopholes in the biosecurity arrangements in the livestock sector may lead to catastrophe in ESBL-related AMR [8]. In fact, ESBL-producing bacteria were identified in meat products, broiler chicken, dairy products, pork etc. [9].

3. Use of Antimicrobials in Food Animals Results

Use of antimicrobials is quite often seen not only while treating or preventing the livestock animals, but also while using as growth promoter. These antimicrobials are many times used in human also. It is known that the antimicrobials used in livestock are hazardous to humans by transmitting resistant bacteria via food chain or through transfer of resistance genes from livestock to human. Therapeutic treatments are done to cure diseased animals. Individual animal may be treated in case of livestock of large size. However, in some other food animals, viz. poultry and fish, mass medication is popular and feasible means of treatment. When seasons are venerable for infectious diseases or before transport or after weaning antimicrobials are used for prophylactic purpose. Sometimes mass-medication procedure, popularly known as metaphylaxis, is carried out to treat sick animals while giving medicines to other animals with the aim of preventing the disease. In such practice of metaphylaxis, drugs at therapeutic dose are applied for brief period [1]. Antimicrobials applied in form of growth promoters are usually given in low concentrations, at the tune of 2.5 to 125 ppm (mg/kg) [10, 11].

To enhance production of livestock, use of antimicrobial is practiced in several countries. For growth promotion and feed efficiency, addition of coccidiostat is not uncommon in broiler ration, several of which are broad antimicrobials, viz. ionophores and sulfonamides. Several other antimicrobials are used in broilers, layers and turkey. These are bacitracin, chlortetracycline, virginiamycin etc. Bacitracin and virginiamycin are used as growth promotion besides for controlling necrotic enteritis and *E. coli* infection. In pig, use of antibiotics is generally practiced for better growth and for prophylactic purposes. Further, antibiotics, e.g. Chlortetracycline, sulfonamides, tiamulin etc. are applied in pig feed after weaning to prevent vulnerable infectious diseases (Table-1). In cattle, antibiotics are used on feedlots to accelerate weight gain, to control liver abscesses, besides for treatment and prophylactic purposes. Monensin, lasolocid, chlotetracyclines are commonly used for these purposes.

Table 1: Use of antimicrobials to promote growth and feed efficiency

Poultry	Pig	Cattle	Mode of application
Chlortetracycline, Tylosin, Bacitracin, Virginamycin, Bambermycin	Chlortetracycline, Erythromycin, Bacitracin, Tiamulin, Tylosin, Virginamycin, Bambermycin	Chlortetracycline, Monensin, Lasalocid, Oxytetracycline, Bacitracin,	Applied to apparently healthy food animals by sub-therapeutic doses (<200 gm per ton for > 2weeks) in groups mostly through feed.

[Adapted from McEwen and Fedorka-Cray, 2002]

Enhanced growth at the tune of 1-11% has shown in the livestock sector. Moreover, reduction in growth of animals after withdrawal of such antimicrobials from feed was also reported. Economic loss in poultry and pork industry of some countries was also observed after banning of over-the-counter sell of antibiotics and as antibiotic growth promoter (AGP).

4. Prevalence of Antibiotic Resistant Bacteria in Studied Animals: A Threat to Food Chain

In the last couple of years (2012 onwards) series of experiments were conducted in the Department of Veterinary Microbiology, WBUAFS on various food animals (poultry birds, goat, pig, buffalo and cattle), reared under various husbandry systems in different agro-climatic zones of West Bengal state, to assess antimicrobial resistance (AMR) pattern besides the probable source of resistance, if any. The observations were varied, however, unique by their own. The results and conclusive remarks drawn out of those studies were narrated in the following section to depict the present scenario of local AMR menace that exists in livestock posing real threat to the food chain, if any.

With the aim of determining prevalence, profiling virulence gene, knowing serotypes and revealing pattern of

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antibiotic resistance of *Salmonella*, an investigation was carried out in backyard poultry reared in the districts of West Bengal. Out of 360 samples tested, 22 Salmonella (6.1%) isolates were generated. Subsequently, those were confirmed using polymerase chain reaction. The virulent *Salmonella* isolates were tested for their antibiotic resistance pattern. The isolates were observed to be resistant to chloramphenicol (100%), gentamycin (100%), norfloxacin (100%), oxytetracyclin (100%), ciprofloxacin (100%), levofloxacin (100%), norfloxacin (100%), tetracycline (100%), erythromycin (75%) and co-trimoxazole (75%). The isolates were sensitive to cephaloridine (100%), ceftriaxone (100%), furazolidone (75%), nalidaxic acid (62.5%), nitrofurantoin (62.5%) and colistin (25%). However, none of the isolates observed to possesses major ESBL-producing genes (bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$) as revealed by PCR. It was observed that in the studied zones, third generation cephalosporin and ampicillins were apparently not used by the farmers in the backyard birds. In this context, it was concluded that backyard birds of West Bengal might be considered as safe food [12].

In another study, assessment was done on the presence of CTX-M-producing *Klebsiella* spp. in indigenous chicken, broiler, and kuroiler birds. The study was carried out in different districts of West Bengal during November 2014 through February, 2015. While assessing type of bacteria phenotypically, the isolates showed *Klebsiella* spp. in 10.7% cases with CTX-M producers. Polymerase chain reaction revealed the isolates had $bla_{CTX\text{-}M}$, whereas 51.5% and 48.5% *Klebsiella* spp. isolates contained bla_{SHV} and bla_{TEM} along with $bla_{CTX\text{-}M}$, respectively. This investigation revealed that the studied birds harbour CTXC-M-producing *Klebsiella* spp. which might be transmitted to the food chain either directly or indirectly [13, 14].

Cloacal swabs (n=202) were collected from ducks of organized farms and backyard to study the prevalence of bacteria of *Enterobacteriaceae* group that produce beta-lactamase. It was observed that, 17.4%, 5.05% and 13.76% isolates contained bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ genes, respectively. Moreover, in the same study, 38.46% Salmonella was isolated. These were found to contain $bla_{CTX\text{-}M}$. *Klebsiella pnemoniae* isolates possessed 33.33% bla_{TEM}, 10.00% bla_{SHV} and 13.33% $bla_{CTX\text{-}M}$. Whereas, 79.82% *E. coli*, 46.15% *Salmonella* and 70% *K. pneumonia* isolates were found to contain bla_{AmpC}. The backyard ducks, reared by marginal farmers in India, cannot afford to use antibiotics in feed or during therapy owing to high cost. This study provided a valuable proof that exposure to contaminated environment might be an additional source for generation of antimicrobial resistant bacteria in backyard ducks [15]. To investigate the prevalence of ESBL/beta-lactamase producing *E. coli* in healthy pigs, 200 rectal swabs were collected from four organized and 10 backyard farms of W.B. Some *E. coli* isolates (n=12) were observed to possess ESBL/beta-lactamase genes studied. The presence of $bla_{CTX\text{-}M\text{-}9}$, $bla_{SHV\text{-}12}$, $bla_{TEM\text{-}1}$ was noticed upon molecular characterization of the isolates. Clonal relationship was detected in two pairs of isolates obtained from organized and backyard farms. The possibility of antibiotic gene transmission farm organized to backyard farm was anticipated. Pigs of backyard farm were suspected to be major source of ESBL/beta-lactamase producing *E. coli* with typical pattern of antibiotic resistance [16].

Prevalence of beta-lactamase / AmpC-producing *Klebsiella* and *E. coli* in organized and backyard healthy pig of West Bengal albeit the pigs had no history of antibiotic intake. Majority of beta-lactamase–producing isolates contained $bla_{CTX\text{-}M\text{-}9}$. Further, 81% *Klebsiella* and all the *E. coli* isolates were having AmpC beta-lactamase ACBL producers. The possible role of contaminated environment as a source of beta-lactamase/ AmpC-producing *Klebsiella* and *E. coli* in pigs was anticipated by the authors [17].

Isolation of enterovirulent *E. coli* (STEC and EPEC) might be an indicator of public health concern as the farmers remain close proximity to goats without maintaining hygienic measures. Moreover, people consume un-pasteurized goat milk and undercooked meat products [18].

ESBL-producing ($bla_{CTX\text{-}M}$, bla_{TEM}, bla_{SHV}) or quinolone resistance gene (qnrA) was not detected in the ETEC isolates of healthy buffalo [19] indicating no use of antibiotics in buffalo husbandry practices.

In an investigation, a total of 50% of the *E. coli* strains detected in the bovine milk samples were found to be positive for ESBL production. The isolates were resistant to the common antimicrobials revealing the gravity of the situation [20].

5. Conclusion

Good animal health and management practices having improved biosecurity measures including vaccine strategies should be encouraged to follow; since animal environment is considered to be an important avenue of generating antimicrobial resistance in food animals. Better growth enhancers in form of natural products, pro- and pre-biotics should be propagated for replacing antibiotic growth promoters to avoid the chance of entering resistant bacteria in food chain. Moreover, monitoring and reporting antibiotic-use at the farmers' level should be executed meticulously to formulate awareness strategies to address the menace of antimicrobial resistance in food chain.

DOI: **10.30726/esij/v8.i1.2021.81005**

Acknowledgements

The author wishes to thank Hon'ble Vice Chancellor, WBUAFS for providing infrastructure and necessary research facilities to the laboratory. The works were financially supported by the Department of Biotechnology, Govt. of India (Grant no. BT/ 164/NE/TBP/2011& BT/PR16149/NER/95/85/2015) and Department of Science & Technology and Biotechnology, Govt. of West Bengal (Grant no. IG-25/2016).

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DOI: **10.30726/esij/v8.i1.2021.81005**