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and Bioremediation Approaches – A Review*

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Application of Antibiotics in Food Animals Production and its Impact on Human Health and Bioremediation Approaches – A Review

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ABSTRACT

Antibiotics applications in food animal production have led to enhance the antibiotics resistant bacteria and transmitted to human through food chain. In globally, India has consumption of 3% antibiotics in food animal production farms, particularly in Mumbai, Delhi and south coasts. It is estimated that in India antibiotics uses in food animal will increase to triple by the year of 2030. Generally, antibiotics are entered into the soil and water bodies by municipal sewage, animal husbandry, pharmaceutical industries, livestock manure and leachate of antibiotics and it easily affects human health through food chains. Antibiotics residues enter into human body by food chain interact with gastrointestinal tract microbiome and cause allergic reactions and develop antimicrobial resistance. This review addresses the antibiotic application in food animal production industry and its impact on human health, and has also suggested some antibiotic biodegradation methods.

Key words: Antibiotics, Biodegradation, Microorganisms, Food animal production, Bioremediation

Predominantly antibiotics have been used for promoting growth and enhance feed proficiency enhancers in food animal production industries in developing countries [1]. Globally more than 50 million kilograms of antibiotics are used in food animal production annually [2]. Worldwide, India has a consumption of 3% antibiotics in food animal production farms, particularly in Mumbai, Delhi and south coasts. It is estimated that in India antibiotics uses in food animal will increase to triple by the year of 2030 [2]. Similarly, in Brazil, Russia, India, China and South Africa antibiotics uses will increase to double in future [1-2]. India, China, Mexico, Iran, US, Argentina, Russia, Spain, Germany and Brazil are top global uses of antibiotics in food animal production. Among them, India will be consuming highest antibiotics in food animal production by 2030 (Fig 1). But now India is in the fourth place among the top 10 countries using of antibiotics in food animal production farm [3]. Food and Drug Administration (FDA) approved the use of antibiotics as supplement with animal

feed in food animal production industries. For example, ampicillin, amoxicillin, cefadroxil, chlortetracycline, doxycycline, erythromycin, flumequine, gentamycin, veneomycin, oxytetracycline, spiramycin, penicillins, sulfadiazine, sulfadimethoxine and tetracyclines are widely used antibiotics in food animal production. However, the world health organization (WHO) has restricted these antibiotics uses in food animal production industry (National Pharmaceutical Regulatory Agency, Malaysia). In India, Food safety and standards authority of India (FSSAI) has banned use of colistin in food animal production [4].

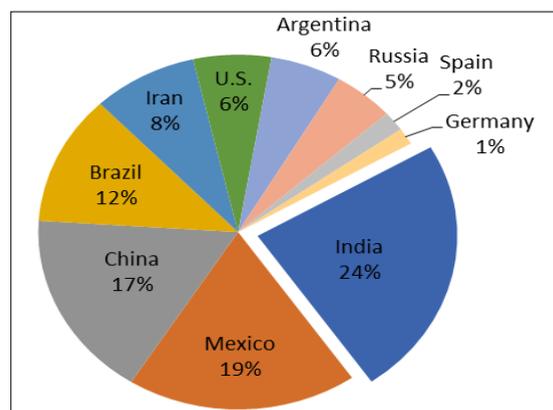


Fig 1 Predicted top 10 countries using antibiotics in food animal productions in 2030

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The use of huge amount of antibiotics in food animal production have led to generation of antibiotics resistance in bacteria which are transmitted to human through food chains and environmental pathway such as contaminated water and soil [5]. This has exhibited the cross resistance with antibiotic uses in clinical treatments and decreasing pathogen resistance in humans [6]. The antibiotics resistance is a most important health risk to animal and human [7-8]. It is estimated that 10 million of death per year world wide by 2050 will be due to the infections with antibiotic resistance bacteria [3]. Recently, there are few data available in India on antibiotics applications in food animal production and its impact on human health and remediation technologies. This review addresses the applications of antibiotics in food animal production industry and its impact on human health, and suggestive measures and methods for some antibiotics waste degradation.

Application of antibiotics in food animals production

Over the year, antibiotics have been used in poultry, dairy, fisheries, cattle and swine farms for therapy, prophylaxis, metaphylaxis, as feed proficiency enhancers and growth promoters [9]. Antibiotic use in food animal production can be grouped into two categories therapeutic and non-therapeutic use. In India, livestock sector plays an important role in socioeconomic and most of the people depend on the livestock sector for their livelihood and income. Also, India is a largest meat, milk and third largest egg producer in the world. It is estimated that in India 7 million tons of meat were produced in 2015-2016 [10]. Although, Food Safety Standards Authority of India (FSSAI) and Export Inspection Council of India (EIC) has not set any antibiotics maximum residues permitted limit in meat and milk products in India, EIC has followed the European Union (EU), Codex Alimentarius Commission (CAC), directive guidelines and maximum residues limit for antibiotics uses in food animal production (Table 1) [11-13]. However, some research observed presence of antibiotic residues in meat and milk products in India. For example, Gaurav *et al.* [14] reported that tetracycline residues in cattle milk sample were collected from Punjab. Out of 133 samples, 18 samples were found to have tetracycline residues in ranges from 16-134.5 µg/L, which is exceeding the maximum residues limit. Similarly, in Punjab 492 milk samples were collected and antibiotic residue analysis were carried out by Moudgil *et al.* [15]. Among 492 milk samples 78 (16%) were found positive for enrofloxacin, oxytetracycline, tetracycline and sulphamethoxazole residues and were above maximum residues limit. In Hyderabad, Sudershan and Bhat [16] reported the presence of oxytetracycline residues in milk. Out of 205 milk samples, 97 samples were found to contain oxytetracycline residues in range from 2 – 6.7 µg/mL. Moharana *et al.* [17] in Chennai, reported that 16.8% of the total milk samples were enrofloxacin positive, among which 8% of the milk sample contained above the maximum residues limit for enrofloxacin residue. In the study carried out by Moudgil *et al.* [18] in Punjab, 4.8% and 3.0% of milk samples were found positive for enrofloxacin (161.2 ng/mL) and oxytetracycline (118.6 ng/mL) residues respectively. Kumarswamy *et al.* [19] in Thrissur, reported that out of 165 milk samples, 4 milk samples were positive for β-lactams, tetracyclines and enrofloxacin, respectively. Lejaniya *et al.* [20] also reported that out 50 milk samples 6 (12%) milk samples were contaminated with both β-lactams,

tetracyclines in Thrissur. Recently, Hebbal *et al.* [21] conducted a study to investigate the presence of oxytetracycline in pooled raw milk samples which were collected in Palakkad. Out of the 215 samples analyzed, 5 (2.33%) samples were positive for oxytetracycline residues (272.11ng/mL). Similarly, in Motihari, Muzaffarpur and Gopalganj districts of Bihar, Ramesh Kumar *et al.* [13] revealed that 3.2% milk samples were positive for enrofloxacin residue. Out of which, 1.2% of milk samples were found to be above maximum residue limit for enrofloxacin residue (125.18µg/kg). On the other hand, Sahu *et al.* [22] detected antibiotics residue in chicken meat at Delhi zone. In this study, totally 70 chicken samples were collected from local markets of Noida, Ghaziabad, Gurgaon, Faridabad and Delhi. Out of 70 chicken samples, 20 samples (28.6%) were positive for Enrofloxacin (131.75 µg/kg) and Ciprofloxacin (64.59 µg/kg) residues respectively. A study was conducted in Kerala, Tamil Nadu, Karnataka and Andhra Pradesh for determining the chloramphenicol, sulphonamide, tetracycline, streptomycin, erythromycin and β-lactam residues in shrimps. Tetracycline, streptomycin and β-lactams were not detected in shrimp, whereas, erythromycin and sulphonamide have been determined in shrimp samples below 100ppb [23]. Based on the above literature survey it is concluded that in India enrofloxacin and oxytetracycline are maximally used in food animal production (Table 2). There is very less number of study was conducted for detection of antibiotics residues in meat and meat products. It is a very urgent need to detect the antibiotics residues in meat and meat product to reduce the antibiotic consumption in food animal production industry. Since, in India antibiotics maximum residues permitted limit in meat and milk products are not regulated and followed according to the CAC guidelines, immediate regulation and containment of antibiotics in food animal production is mandatory to prevent the future generation from the threat of antibiotic resistance.

Table 1 Maximum residues limit (MRL) of antibiotic uses in food animal production

Antibiotics	MRL in meat (µg/kg)	MRL in milk (µg/L)
Ampicillin	-	4
Amoxycillin	50	4
Benzyl penicillin	50	4
Cloxacillin	-	30
Chlortetracycline	200	100
Ceftiofur	1000	100
Dicloxacillin	-	100
Dihydrostreptomycine	600	200
Gentamycine	100	200
Enrofloxacin	-	100
Oxacillin	-	30
Oxytetracycline	200	100
Streptomycin	200	-
Sulphonamides	-	100
Sulfadimidine	-	25
Tetracycline	200	100
Tilmicosin	100	-
Tylosin	100	-

Table 2 Document evidence of antibiotics consumption in food animal production in India

Antibiotics	Sample	Concentration	Place	References
Tetracycline	Milk	134.5µg/L	Punjab	[14]
Oxytetracycline	Milk	6.7 µg/mL	Hyderabad	[16]
Enrofloxacin	Milk	161.2ng/mL	Punjab	[15]
Oxytetracycline	Milk	118.6ng/mL	Punjab	[18]
Oxytetracycline	Milk	272.11ng/mL	Palakkad	[21]
Enrofloxacin	Milk	125.18µg/kg	Bihar	[13]
Enrofloxacin	Milk	58.70ppm/mL	Chennai	[17]
Enrofloxacin	Chicken liver	131.75µg/kg	Delhi	[22]
Ciprofloxacin	Chicken liver	64.59µg/kg	Delhi	[22]
Erythromycin	Shrimps	61.12ppb	Andhra Pradesh	
Erythromycin	Shrimps	42.06ppb	Kerala	
Erythromycin	Shrimps	45.13ppb	Karnataka	[23]
Sulfonamides	Shrimps	56.91ppb	Andhra Pradesh	
Sulfonamides	Shrimps	44.00ppb	Kerala	
Sulfonamides	Shrimps	55.10ppb	Tamil Nadu	
Sulfonamides	Shrimps	65.60ppb	Karnataka	

Toxicology effects of antibiotics on human health

The extensive application of antibiotics in food animal production poses a serious threat to human health and natural microbial systems throughout the world. However, nowadays there is no quantitative model for suitable measure of antibiotics resistance risk in environment [24]. As per the Centers for Disease Control and Prevention (CDC) reports the antimicrobial resistance is a most responsible entity for 48,000 deaths annually in U.S. and European Union [25]. Generally, antibiotics enter into the soil and water bodies by municipal sewage, animal husbandry, pharmaceutical industries, livestock manure and leachate of antibiotics and it easily affects human health through food chains [24-26]. Antibiotics residue enter into human body by food chain and interact with gastrointestinal tract microbiome and causes allergic reactions and develops antibacterial resistance [27-28]. Cox *et al.* [29] observed that antibiotics residues induced changes in gastrointestinal microbiota by epidemiological and clinical investigation. Similarly, a high concentration of nitrofurans causes carcinogenic effect [30]. Therefore, health organizations like WHO, CDC, American Medical Association banned certain antibiotics used in food animal production. Furthermore, in India food safety and standards authority of India has banned carbadox, chloramphenicol, colistin, metronidazole and nitrofurans uses in food animal production.

Bioremediation of antibiotics contamination

Antibiotics widely used for prevention and treatment of diseases in animal and humans. Furthermore, antibiotics are used in food animal production such as aquaculture, poultry, and veterinary, and in agriculture [31]. Partially or metabolized antibiotics are resealed into environment by urine and faces of animal excretion from municipal sewage, pharmaceutical industries, livestock manure [32]. The presence of antibiotic residues in the environment can create antibiotic resistant bacteria in environmental

ecosystem [31]. Moreover, antibiotic contamination in soil can affect the soil microbiota and biogeochemical cycle [33]. Sengupta [34] described that antibiotic contaminated water can cause allergy and discoloration of teeth in human and animals. The antibiotic contamination in environment is a major threat to all living organisms. Therefore, it is an emergency need to search a technology for degradation or removal of the antibiotic contamination from the environment. Recently, various techniques such as photocatalytic degradation, [35] adsorption [36], chemical oxidation [37] and bio-electrochemical [38] have been used for removal of antibiotic contamination in environment. These techniques have many disadvantages like generation of secondary toxic materials, high cost and incompetence [31]. Bioremediation is an alternative method for these techniques, because bioremediation is cost effective, ecofriendly sound and promising method for remediation of antibiotics contamination. Bioremediation is utilization of microorganisms (bacteria, fungi and algae) to breakdown and degrade toxic molecules into non/less toxic molecules [39]. Recently, many scientists have identified different microbial floras like bacteria, fungi and algae for degradation of antibiotics contamination in environment. For example, Sodhi *et al.* [31] reported that *Alcaligenes sp.* MMA have ability to remove high concentration (84%) of amoxicillin at 14 days treatment in M9 minimal media. Similarly, Yang *et al.* [40] have isolated SF1 (*Pseudomonas sp.*), A12 (*Pseudomonas sp.*), strains B (*Bacillus sp.*), and SANA (*Clostridium sp.*) from sludge for degradation of oxytetracycline, tetracycline, chlortetracycline, amoxicillin, sulfamethazine, sulfamethoxazole, sulfadimethoxine under the both anaerobic and aerobic condition. It was found that strain B effectively degraded the amoxicillin (89.1%), chlortetracycline (81.6%) and sulfamethoxazole (95.1%). Additionally, strain SANA degraded the amoxicillin (81.4%), chlortetracycline (59.4%) and sulfamethoxazole (89.6%) after 15 days of treatments. Liu *et al.* [6] separated

a gentamicin degrading bacteria (AMQD4-*Providencia vermicola*, *Brevundimonas diminuta*, *Alcaligenes sp.* and *Acinetobacter*) from biosolid waste. Among them AMQD4 and *Brevundimonas diminuta* have great ability to degrade highest gentamicin (50%) in sewage. It has been showed that *Raoultella sp.* XY-1 and *Pandoraea sp.* XY-2 bacterial consortium effectively degraded tetracycline (81.72%) at 12 days in lysogeny broth medium [41]. In other study, Yin *et al.* [42] isolated a tetracycline degrading bacteria TR5 (*Klebsiella pneumoniae*) from chicken manure. It was found that TR5 strain efficiently degraded tetracycline (90.0%) within 36 h. Moreover, Wen *et al.* [43] showed that

Escherichia sp. and *Candida sp.* effectively degrade the doxycycline (92.52% and 91.63%) after 7 days in graded media. Bioremediation with fungi (mycoremediation) is an effective and emerging method to degradation of antibiotics, because some fungi use antibiotics as nutrient and energy sources during metabolic processes [33]. For example, Singh *et al.* [44] reported that white rot fungi *Pleurotus ostreatus* effectively degraded the ciprofloxacin 95.07% after 14 days of treatment. The results of the study by Gou *et al.* [45] showed that *Phanerochaete chrysosporium* degraded maximum percentage of sulfamethoxazole (74%) after 10 days of treatment in liquid medium.

Table 3 Antibiotics degrading microorganisms (bacteria, fungus and algae)

Microorganisms	Antibiotics	Degraded (%)	Treatment duration	References
Bacteria				
<i>Alcaligenes sp.</i> MMA	Amoxicillin	84	14 days / M9 minimal	[31]
SF1 (<i>Pseudomonas sp.</i>)	Chlortetracycline, Amoxicillin and Sulfamethoxazole	81.6, 89.1 and 95.9	8 days/sludge	[40]
A12 (<i>Pseudomonas sp.</i>)	Chlortetracycline, Amoxicillin and Sulfamethoxazole	89.4, 93.4 and 99.3	8 days/sludge	[40]
Strains B (<i>Bacillus sp.</i>)	Amoxicillin, Chlortetracycline and Sulfamethoxazole	89.1, 81.6 and 95.1	15 days/Sludge	[40]
SANA (<i>Clostridium sp.</i>)	Amoxicillin, Chlortetracycline and Sulfamethoxazole	81.4, 59.4 and 89.6	15 days /Sludge	[40]
<i>Raoultella sp.</i> XY-1 and <i>Pandoraea sp.</i> XY-2	Tetracycline	81.72	12 days/lysogeny broth medium	[41]
TR5 (<i>Klebsiella pneumoniae</i>)	Tetracycline	90.0	36 h	[42]
<i>Escherichia sp.</i>	Doxycycline	92.52 and 91.63	7 days/ graded media	[43]
AMQD4 and <i>Brevundimonas diminuta</i>	Gentamicin	50.0	7 days/sewage	[6]
Fungus				
<i>Pleurotus ostreatus</i>	Ciprofloxacin	95.07	14 days	[44]
<i>Candida sp.</i>	Doxycycline	91.63	7 days/ graded media	[43]
<i>Phanerochaete chrysosporium</i>	Sulfamethoxazole	74.0	10 days	[45]
<i>Trametes versicolor</i> and <i>Ceriporia lacerate</i>	Streptomycin	60.65 and 50.05	14 days	[45]
Algae				
<i>C. pyrenoidosa</i> and <i>M. aeruginosain</i>	Cefradine	42.63 and 39.11	24h	[48]
<i>C. pyrenoidosa</i> and <i>M. aeruginosain</i>	Amoxicillin	71.25 and 62.92	24h	[48]
<i>Chlorella vulgaris</i>	Levofloxacin	12.0	11 days	[47]
<i>Scenedesmus obliquus</i> and <i>Chlamydomonas Mexicana</i>	Carbamazepine	35 and 28	10 days	[47]

Moreover, a study has showed that *Trametes versicolor* and *Ceriporia lacerata* removed 60.65 and 50.05% of streptomycin in MEB medium after 14 days of 400 ppm streptomycin treatment [46]. On the other hand, Xiong *et al.* [47] investigated the biodegradation of levofloxacin by six microalgae species including *Chlorella vulgaris*, *Ourococcus multispurus*, *Chlamydomonas pitschmannii*, *Micractinium resseri*, *Chlamydomonas Mexicana*, *Tribonema aequale*. Among the six microalgae species, *Chlorella vulgaris* removed highest percentage of levofloxacin (12% of LEV at 1 mg L) in medium within 11 days. In another study, Xiong *et al.* [47] evaluated that carbamazepine tolerance and biodegradation efficiency in *Scenedesmus obliquus* and *Chlamydomonas mexicana*. It

was found that the both microalgae species have carbamazepine tolerance ability and maximum degradation (35% and 28%). Du *et al.* [48] carried out an influence of light intensity on microalgae growth and antibiotics removal in growth medium. It was found that within 24h treatment (42.63% and 71.25%) the cefradine and amoxicillin were removed by *Chlorella pyrenoidosa*. Similarly, *Microcystis aeruginosain* removed cefradine 39.11% and amoxicillin 62.92%, respectively. When compared to *M. aeruginosain*, *C. pyrenoidosa* have great efficiency in removal of both antibiotics within 24h. All the above studies indicate the promising potential of identified microorganisms including bacteria, fungus and algae to have great ability in removing of antibiotics from contaminated environment.

CONCLUSION

This review reviewed the antibiotics residues presence in milk and meat, and the bioremediation techniques for antibiotics contamination removal. The residual concentration of antibiotics is increased in environment due to the large application of antibiotics in

food animal production and other uses. Therefore, it is necessary to monitor the presence of antibiotics residues levels in meat, milk and urine with in regulatory limits to for minimize the antibiotics contamination in environment. Moreover, it is an urgent necessity to develop an efficient, ecofriendly and cost-effective technology for removal of antibiotics contamination in environment.

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