



## STUDY OF ANTIBIOTIC RESISTANCE IN BACTERIA ISOLATED FROM TABLE EGG

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### ABSTRACT

A total of 132 egg samples were collected from different areas of Jaipur, India. Gram negative enterobacteria were isolated initially on Nutrient agar and then on selective-differential media and further characterized by different biochemical tests. Commonly prescribed antibiotics in patients for gastro-intestinal infection were used for antibiotic susceptibility test. Against different antibiotics different resistance pattern were found ( $p < 0.01$ ). The highest resistance rate were detected against Cefixime (86.66%) whereas highest sensitivity rate (100%) were recorded against Gentamicin, Levofloxacin and Ciprofloxacin. Also, most of the isolates (73.3%) were found to be multi drug resistant as these showed resistance against three or more antibiotics tested. Multiple antibiotic resistance index of isolated microbes from table egg ranged from 0.10 to 0.70. It can be concluded that commercial eggs which are consumed as food may harbour multi-drug resistant bacterial pathogens and if consumed raw may cause severe ailments in consumers. Antibiotic resistance of bacterial species will make the clinical treatment of diseases more difficult. Thus aim of the present study is to assess the risk of antibiotic resistance in table eggs and egg products.

**KEYWORDS:** Antibiotic, Susceptibility, Enterobacteriaceae, Multi-drug resistance.



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## INTRODUCTION

Eggs which are consumed world-wide, can be part of a healthy diet as these are considered as the most nutritious, economic source of protein, lipids, vitamins, and minerals.<sup>1</sup> In India, the consumption of Eggs reveals wide inter-state variations, with the consumption index varying from lowest in Gujarat (28.2) and highest in West Bengal (286.5) in year 2004-05. The consumption index of Jaipur, Rajasthan is second lowest in the country.<sup>2</sup> But the egg consumption have increased extensively during the last few decades. As egg consumption per capita was registered a significant growth increasing from about 6 eggs per year in 1987-88 to 21 eggs in 2009-10 in rural area and from 17 eggs per year in 1987-88 to 32 eggs in 2009-10 in urban area.<sup>3</sup> This increased consumption of egg is also the efforts of National Egg Co-ordination Committee (NECC), Govt. of India, which promotes egg as complete food.<sup>4</sup> The presence of nutrient substances in the egg creates an appropriate environment for the development of bacterial microbiota, including pathogenic bacteria thus making eggs a potential pathogens. The consumption of infected eggs (raw or uncooked) may cause different food-borne diseases which can be treated only by the use of chemotherapeutic agents. Many findings suggest that inadequate selection of antimicrobials may lead to resistance in various bacteria and make the treatment more difficult in human and animals. Medical treatment of these infections has been gradually complicated by the occurrence of resistance to most first-line antibiotics. These resistant bacteria could pass to human through the food chain<sup>5</sup>, thus producing resistance against particular antibiotics. In animals, antibiotics are used in livestock production, disease prevention and growth promoters. The use of these antibiotics in animals disrupts normal intestinal microbiota, resulting in the emergence of antibiotic-resistant microbes.<sup>6</sup> Different studies have been reported on antimicrobial resistance of bacteria and many of the resistant bacteria recovered from poultry or poultry-related samples particularly include *Salmonella*, *Escherichia coli*, other *Enterobacteriaceae* and *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Shigella dysenteriae* etc.<sup>7-20</sup> Poultry production is commonly associated with large scale use of antibiotics and is therefore considered as one of the major source of new combinations of antibiotic resistance. In poultry industry, the antimicrobial have been used at subtherapeutic or therapeutic doses to enhance the growth, feed conversion efficiency and for prevention and control of diseases.<sup>21</sup> Most commonly used antibiotics in poultry industry are Penicillins, Cephalosporins, Sulfa, Macrolides, Tetracyclines and Ionophores etc. The indiscriminate use of antibiotics in poultry has resulted in development of resistance to some of these antibiotics used in the treatment of infectious/food-borne diseases. Unluckily, the development of bacterial resistance to antibiotics quickly reduced ability of a particular antibiotic, so it is necessary to test pathogens against various antibiotics to determine susceptibility or resistance to that drug. This study was undertaken to investigate the antibiotic resistance against bacterial isolates from eggshell and

inner content of egg collected from retail outlets of Jaipur, Rajasthan, India.

## MATERIALS AND METHODS

### **Sample Collection and Sample enrichment**

A total of 132 egg samples were collected from different areas in Jaipur city, India during summer season. The egg samples were collected in sterile bags and transported to laboratory and processed within six to eight hours of collection. For egg-shell sampling, sterile cotton swabs dipped in sterile buffered peptone water (BPW) were used to swab the entire surface area of egg shell. The swabs were directly inoculated into 10 ml BPW in screw-capped bottles and subsequently incubated at 37°C for 24 hours.<sup>4</sup> For egg albumin and yolk sampling, outer surface of the eggs were disinfected (by wiping with surgical gauze soaked in 70% ethanol) and opened around the air sac area with a sterile forceps. The albumin and yolk content of all five eggs were pooled and homogenized to make one sample of albumin and yolk, respectively. The homogenized samples of albumin and yolk were serially diluted in normal saline till 10<sup>-3</sup> dilution and then incubated at 37°C for 24 hours.

### **Isolation and Characterization of Bacteria**

The processed samples were then used for the isolation of Gram Negative members of Enterobacteriaceae. Isolation was initially carried out on Nutrient agar and then on selective and differential media (i.e. MacConkey agar, EMB agar, Bismuth sulphite agar, SS agar and XLD agar) and incubated at 37°C for 24-48 hours.<sup>22</sup> Bacterial isolates that showed growth on selective and differential medium were then identified by gram's staining and later on characterized by biochemical tests like oxidase test, catalase test, starch hydrolysis, casein hydrolysis, indole test, Methyl-red and Voges-proskauer test (MR-VP), citrate utilization test, H<sub>2</sub>S production test, Kligler iron agar test.<sup>23</sup>

### **Antimicrobial Susceptibility Test**

The antibiotic susceptibility pattern of Enterobacteria isolates were performed using agar disc diffusion technique as described by Kirby-Bauer.<sup>24</sup> Isolated bacterial colonies were mixed in normal saline and turbidity was matched with 0.5 McFarland turbidity standards.<sup>25</sup> The antibiotic sensitivity test was performed using Mueller-Hinton agar plates and antibiotic discs of ten commonly prescribed chemotherapeutic agents/antibiotics [i.e. Cifoxitin (CX<sup>30</sup>), Azithromycin (AZM<sup>15</sup>), Amoxycillin (AMX<sup>10</sup>), Gentamicin (GEN<sup>10</sup>), Cefixime (CFM<sup>5</sup>), Levofloxacin (LE<sup>5</sup>), Vancomycin (VA<sup>30</sup>), Ciprofloxacin (CIP<sup>30</sup>), Tetracyclin (TE<sup>30</sup>), Amoxyclave (AMC<sup>30</sup>)] used for gastro-intestinal infection. The selected antibiotic discs were placed on Mueller Hinton agar plates inoculated with bacteria and incubated at 37°C for 24 hours. The organisms were observed for antibiotic sensitivity based on diameter of inhibition zones produced and results were interpreted as per standard of CLSI (Clinical and Laboratory Standards Institute).<sup>26</sup> The collected data was analyzed with the SPSS software. MAR (Multiple antibiotic resistance) index would provide useful information for the evaluation of a health risk.<sup>27,28</sup> MAR index value

higher than 0.2 is considered to have originated from high-risk source of contamination such as human, commercial poultry farms, swine and dairy cattle where antibiotics are often used.

## RESULTS AND DISCUSSION

The contamination generally occurs through the shell but temperature, storage time and other environmental factors make the condition favourable for migration of bacteria from the surface of the shell to the inner contents of the egg.<sup>29</sup> In the present study, out of the total 132 samples examined, 38 (28.7%) were found to be infected. Of the 38 isolates, 32 (24.2%) were from eggshell and 4 (3%) from albumin and 2 (1.5%) from yolk, thus showing that the risk of infection by micro-organisms present on egg shell (24%) was higher than that of albumin and yolk (4%). Adesiyun also reported that the rate of contamination was higher on eggshell (28.3%) than on egg albumin (4%) and egg yolk (3%) whereas Arathy detected only 8% contamination from shell and 5% from yolk samples.<sup>21,30</sup> And Saitanu also

isolated *E.coli* at lower level as 3.5% from egg shells and 1.2% from inner contents.<sup>31</sup> In this study, out of 38 bacterial isolates, only 15 isolates showed growth on selective and differential medium which were further characterized by different biochemical tests (Table 1). The biochemical tests thus performed showed that the isolates may belong to the following genus<sup>23</sup>; *Citrobacter spp.*, *Enterobacter spp.*, *Escherichia spp.*, *Klebsiella spp.*, *Proteus spp.*, *Shigella spp.* and *Serratia spp.* whereas Nazer have isolated *Streptococcus spp.*, *Bacillus spp.*, *Staphylococcus spp.*, *Klebsiella spp.*, *Enterobacter spp.*, *Escherichia coli*, *Citrobacter spp.*, *Proteus spp.* and *Pseudomonas spp.* from the eggs in Iran<sup>8</sup>; Musgrove identified isolates as *Cedecea*, *Citrobacter*, *Erwinia*, *Hafnia*, *Klebsiella*, *Kluyvera*, *Leclercia*, *Morganella*, *Proteus*, *Providencia*, *Rahnella*, *Salmonella*, and *Serratia*<sup>32</sup>; from which most of the isolates were members of the family Enterobacteriaceae<sup>5,33</sup>, thus showing the rate of contamination is higher with gram negative Enterobacteriaceae than gram positive bacteria.

**Table 1**  
**Biochemical characterization of isolates**

Sample	Oxidase test	Catalase test	Indole Production	MR Reaction	VP Reaction	Citrate Utilization	Casein Hydrolysis	Starch Hydrolysis	Lactose fermentation	Glucose fermentation	H <sub>2</sub> S production	Gas production
MLP2	-	+	+	+	-	-	-	-	-	-	-	-
KLR1	-	+	+	+	-	-	-	-	-	+	-	-
KLR2	-	+	-	+	-	-	-	-	-	+	-	-
KLR4	-	+	+	+	-	-	-	-	-	+	-	-
HSP1	-	+	+	+	-	-	-	-	+	+	-	-
MNS4	-	+	+	+	-	-	-	-	-	+	-	-
StR2	-	+	-	-	-	-	+	-	-	-	-	-
CND1	-	+	-	-	-	+	+	-	-	+	-	-
CND2	-	-	-	+	-	+	+	+	-	+	-	-
AMR3	-	+	-	+	-	+	-	-	-	+	-	+
BRM4	-	+	-	-	-	+	+	+	-	+	-	+
SMS1	-	+	-	+	+	-	-	-	-	+	-	+
SMS3	-	+	-	-	-	+	-	-	+	-	-	-
MNS(A) 10 <sup>-1</sup>	-	+	-	+	-	-	-	-	-	+	-	-
SMS(A) 10 <sup>-1</sup>	-	+	-	-	-	+	+	-	+	+	-	-

\* (+) = positive test, (-) = negative test, (MLP2, KLR1, KLR2, KLR4, HSP1, MNS4, StR2, CND1, CND2, AMR3, BRM4, SMS1, SMS3, MNS(A)10<sup>-1</sup>, SMS(A)10<sup>-1</sup> = Samples coding)

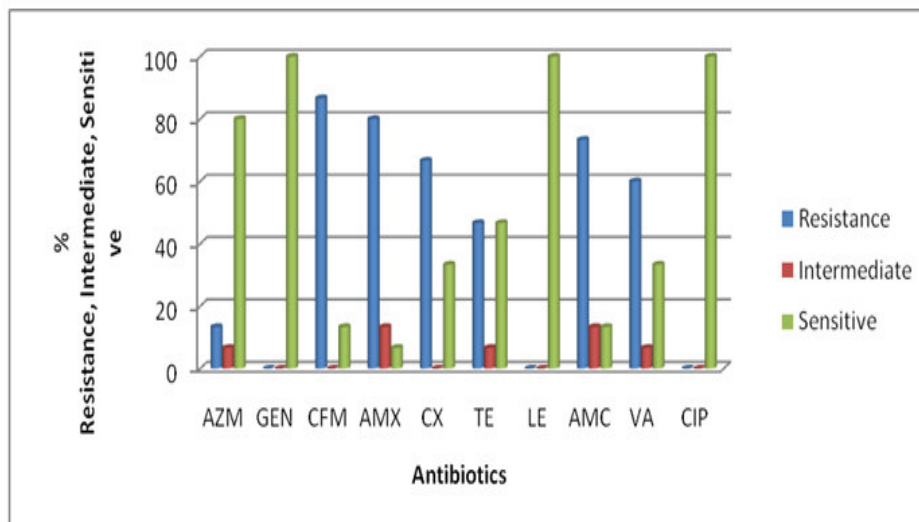
The antibiotic susceptibility of the isolates against different antibiotics is shown in Table 2 and Histogram 1. Among all the antibiotics tested all the bacterial isolates were highly sensitive to Gentamicin, Levofloxacin and Ciprofloxacin with maximum zone of inhibition 27mm, 38mm and 38mm respectively whereas some of the bacterial isolates were sensitive to Cifoxitin, Tetracycline and Vancomycin with 35mm, 31cm and 28mm as their maximum zone of inhibition. Among the bacterial isolates only two samples viz. KLR1 and KLR2

were sensitive to Cefixime with 41mm and 22mm as their zone of inhibition, whereas Amoxicillin and Amoxycylave was found sensitive for samples SMS3 and MNS(A)10<sup>-1</sup> with 21mm and 26mm as their zone of inhibition. Multi drug resistance (MDR) was observed in 73.33% of the isolates. The highest resistance rates were recorded against Cefixime (86.66%), Amoxicillin (80%) and Amoxycylave (73.33%). While the highest sensitivity rate (100%) were recorded against Gentamicin, Levofloxacin and Ciprofloxacin.

**Table 2**  
**Antibiotic Susceptibility test of bacterial isolates**

Antibiotic group	Antibiotic	Diffusion zone breakpoint (mm)	Sensitive (%)	Intermediate (%)	Resistant (%)	P value
Macrolides	Azithromycin	≤13	80	6.66	13.33	0.0013
Aminoglycoside	Gentamicin	≤12	100	0	0	
Tetracyclines	Tetracyclin	≤11	46.66	6.66	46.66	
Glycopeptide	Vancomycin	≤14	33.33	6.66	60	
β-Lactams	Amoxicillin	≤13	6.66	13.33	80	
β-Lactams	Amoxyclave	≤13	13.33	13.33	73.33	
Cephalosporin	Cifoxitin	≤14	33.33	0	66.66	
	Cefixime	≤15	13.33	0	86.66	
Quinolones	Ciprofloxacin	≤15	100	0	0	
	Levofloxacin	≤13	100	0	0	

\*mm = millimeter



**Histogram 1**  
**Antibiotic susceptibility profile**

**Table 3**  
**MAR index and resistance pattern of the isolates**

Isolate	MAR index	Resistance Profile
MLP2	0.4	AZM CFM AMX AMC
KLR1	0.1	AMC
KLR2	0.5	AMX CX TE AMC VA
KLR4	0.6	CFM AMX CX TE AMC VA
HSP1	0.4	CFM AMX CX AMC
MNS4	0.2	CFM AMX
MNS(A)10 <sup>-1</sup>	0.1	CFM
StR2	0.6	CFM AMX CX TE AMC VA
CND1	0.6	CFM AMX CX TE AMC VA
CND2	0.7	CFM AMX CX TE AMC VA
AMR3	0.4	CFM AMX CX VA
BRM4	0.6	CFM AMX CX TE AMC VA
SMS1	0.5	CFM AMX CX AMC VA
SMS3	0.1	CFM
SMS(A)10 <sup>-1</sup>	0.6	CFM AMX CX TE AMC VA

\*Cifoxitin = CX, Azithromycin = AZM, Amoxicillin = AMX, Gentamicin = GEN, Cefixime = CFM, Levofloxacin = LE, Vancomycin = VA, Ciprofloxacin = CIP, Tetracyclin = TE, Amoxyclave = AMC

In the present study, all the isolates were susceptible against gentamycin which is showing similarity with the results of Talebian<sup>34</sup>; lowest resistance rate obtained probably because of its low consumption on poultry due to its very low absorption by the digestive system of poultry birds. Whereas Kuljinder reported resistance at higher level i.e. 80%.<sup>35</sup> The resistance rate against tetracycline (46.66%) was similar to the result of

Muhammad who reported approximately 52% however higher resistance rate was reported by Samah who recorded resistance rate 92.9%.<sup>1,19</sup> The results obtained for azithromycin and levofloxacin were different from the results obtained by Kuljinder who recorded a higher resistance rate of 88% & 54.4% respectively.<sup>35</sup> The resistance rate against ciprofloxacin (0.00%) was in accordance with results of Suleiman whereas Sultana

reported resistance at higher level i.e. 54.4%.<sup>36,37</sup> The resistance rate against vancomycin (60%) was in accordance with the result of Manie who reported a rate of approximately 62%.<sup>38</sup> And higher resistance rate was recorded by Harsha who recorded resistance level at 93.9%.<sup>4</sup> The results obtained for amoxicillin (80%) was different from the results observed by Sheikh who recorded resistant at higher level 92.86% whereas Motayo found the resistant rate at lower level 16.8%.<sup>39,40</sup> Jensen & Hammerum and Belmar-Liberato reported 100% sensitivity against amoxyclove but Pedersen reported all the isolates were resistant.<sup>41,42,43</sup> In the present study, an increasing frequency of amoxyclove-resistant micro-organisms (73.33%) was observed. The resistance rate against cefixime (66.66%) was agreed with the result of Murgrave who recorded resistance rate 61%.<sup>18</sup> This study showed the higher resistance rate against cefixime 86.66% which is not similar with the results of Salehi, Muhammad and Sultana who detected resistance rate 14%, 68% and 72% respectively.<sup>19,37,44</sup> Isolates showing resistance to three or more tested antibiotics were considered to be multi-drug resistant (MDR).<sup>21</sup> In our study, the results showed that 73.33% isolates were multi-drug resistant as these showed resistance to three or more antibiotics; from which approximately 66% isolates were from egg shell and 7% isolates were from egg albumin. Approximately 53% of the isolates were found to be resistant to five or more antibiotics tested in this study. Samah also reported 94% of *E.coli* isolates were resistant to five and more antimicrobial drugs.<sup>1</sup> In the present study, Gentamicin, ciprofloxacin and levofloxacin works much better ( $p < 0.05$ ) than other antibiotics used, so there is no

significant difference between these three antibiotics. The MAR index of isolated microbes from table egg ranged from 0.10 to 0.70.

## CONCLUSION

The present study thus concludes that the commercial eggs which are consumed as food may harbour bacterial pathogens and generated baseline data for multidrug resistant bacteria. Since the incidence of resistance against antibiotics is different and increasing, the use of antibiotics must be limited in poultry farms in order to reduce the development of antibiotic resistance. In order to assess the risks of antimicrobial resistance occurring in eggs, eggs products and other food and its potential transfer to humans, it is essential to minimize the misuse of antimicrobials in different industries (poultry and medicine) and to monitor antimicrobial resistance time to time.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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