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## Antimicrobial resistance on bacteria from the gastrointestinal tract of chicken

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### Abstract

The study was carried out to isolate and identify bacteria from gastro-intestinal tract infections in chicken. The isolates were subjected to antibiogram studies. The samples comprised 50 swabs from the enteric tract of dead chicken with a history of diarrhoea and were brought for post-mortem examination to the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy. Out of the 72 isolates obtained, 43 were identified as *Escherichia coli* along with seven *Klebsiella pneumoniae*, three *Salmonella paratyphi A*, 11 *Proteus spp.*, three *Citrobacter spp.* and five *Staphylococcus aureus* based on colony morphology, Gram staining and various biochemical tests. Antibiotic resistance was detected against commonly used antibiotics using the Kirby-Bauer disc diffusion method. Most of the isolates exhibited a high degree of resistance to antibiotics such as clindamycin and methicillin and varying degrees of susceptibility to other antibiotics.

**Keywords:** *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella paratyphi A*, gastro-intestinal tract infections, chickens

### Introduction

Bacterial gastrointestinal tract infections are most common in chickens. The gastrointestinal tract of the chicken plays an important role in the digestion and absorption of nutrients. It is densely populated with different microorganisms like bacteria, fungi, protozoa, archaea and viruses, among which bacteria are dominant (Shang *et al.*, 2018) <sup>[1]</sup>. Moreover, 640 different species isolated represent previously unknown bacterial genera extracted from the community samples (Apajalahti *et al.*, 2004) <sup>[2]</sup>. Gastrointestinal tract of chicken may get infected usually between the age of 20-30 days due to various factors, viz. wet litter, non-specific enteritis, poor weight gain, decreased nutrient digestibility and reduced absorption of required nutrients (Ranjitkar *et al.*, 2016) <sup>[3]</sup>.

Bacteria commonly present in the enteric tract of chicken include *Escherichia coli* (*E. coli*), *Klebsiella spp.*, *Proteus spp.*, *Salmonella spp.*, *Campylobacter spp.* and *Staphylococcus spp.* (*S. aureus*). All these organisms are opportunistic pathogens. The main gastrointestinal tract infections in chicken are salmonellosis, colibacillosis, *Staphylococcus aureus* infection and necrotic and ulcerative enteritis. These ailments affect the health of birds thereby causing heavy economic loss to the farmers.

The inadvertent use of antibiotics in this sector resulted in the development of severe antibiotic resistance in the bacterial species.

### Materials and Methods

Samples from the gastrointestinal tract were collected from 50 dead chickens brought for post-mortem examination to the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur. The swabs collected from the enteric site of the chicken were directly inoculated on Tetrathionate broth (TTB) and were incubated at 37°C for 24-48 h. The culture from TTB was sub-cultured in Mac Conkey agar (MCA), Blood agar (BA), Xylose- Lysine Deoxycholate agar (XLD) and Eosin Methylene Blue agar (EMB) for further identification.

The cultural characters obtained on different media were observed. The isolates were subjected to Gram staining to observe the morphology and staining characteristics (Koneman *et al.*, 1983; Quinn *et al.*, 1994)<sup>[4, 5]</sup>. Further, the isolates were then identified by biochemical tests Barrow and Feltham (1993)<sup>[6]</sup>.

Antibiogram was performed with 14 antibiotics by disc diffusion method as per Bauer *et al.* (1966)<sup>[7]</sup>. Amoxicillin/clavulanic acid 30µg (AMC), ceftriaxone/tazobactam 10µg (CIT), ceftriaxone/sulbactam 30µg (CIS), ciprofloxacin 30µg (CIP), cefotaxime 30µg (CTX), clindamycin 2µg (CD), enrofloxacin 10µg (EX), ertapenem 10µg (ETP), gentamicin 10µg (GEN), imipenem 10µg (IMP), methicillin 30µg (MET), meropenem 10µg (MRP), norfloxacin 10µg (NX), ofloxacin 2µg (OF). The zones of inhibition were measured in mm and interpreted.

## Result and Discussion

Samples were collected from 50 dead chickens, in tetrathionate broth and blood agar 72 isolates were obtained. Preliminary identification of the pure cultures of the isolates obtained on MCA and BA based on colony morphology, Gram's reaction and biochemical characters. On Gram staining, five were Gram-positive cocci and 67 isolates were Gram-negative bacilli.

There were 42 isolates confirmed as *E. coli* (Fig.1) Eight isolates were observed as *Klebsiella pneumoniae* (Fig.2) and Three isolates were detected as *Salmonella paratyphi A* (Fig.3). Eleven isolates were confirmed as *Proteus mirabilis*, three isolates were confirmed as *Citrobacter freundii* and five isolates were confirmed as *Staphylococcus aureus* (Fig.4). Confirmation of these organisms based on colony characteristics and biochemical identifications. The results of the antibiogram performed with 14 antibiotics were demonstrated (Table.1).

Majority of the isolated organisms during the present study belonged to *Enterobacteriaceae* family of which, more than 50 per cent were *E. coli*. More or less similar observations were recorded by Sooryan (2019)<sup>[8]</sup>, Sebastian *et al.* (2021)<sup>[9]</sup> and Islam *et al.* (2014)<sup>[10]</sup>. Thus the observation that *E. coli* were the major commensals in the gut of chickens, while they do have the ability to become pathogenic when favourable situations arose, was in conjunction with the observations of other researchers in the same field.

Isolates that exhibited pink colonies on Mac Conkey agar with IMViC -- ++ were identified as *K. pneumoniae* (around 10 per cent) in the study which had potential to end up in a zoonotic disease. Dandachi *et al.* (2018)<sup>[11]</sup>, Tantawy *et al.* (2018)<sup>[12]</sup> and Tawab *et al.* (2018)<sup>[13]</sup> reported the existence of *K. pneumoniae* as a prominent bacterial organism in the GI tract of chicken.

Non-lactose fermenting isolates (IMViC -+--) which did not produce a black colour colony in XLD agar (without H<sub>2</sub>S production) were identified as *Salmonella paratyphi A*. Three of the total 72 isolates 4.16 per cent were identified as *Salmonella*. Reports of Tawab *et al.* (2018)<sup>[13]</sup>, Akbar and Anal (2013)<sup>[14]</sup> and Anju *et al.* (2014)<sup>[15]</sup> revealed isolation of *Salmonella spp.*, where Anju *et al.* (2014)<sup>[15]</sup> recorded 4.44 per cent isolates of *Salmonella spp.* Thus the present study established similar results as that of other researchers.

Those non-lactose fermenting isolates (IMViC -+--) which produced black colour colonies on XLD agar were identified as *Proteus spp.* (15.2 per cent of the total isolated bacteria) from the GI tract of chicken. Nemati (2013)<sup>[16]</sup> and Dandachi *et al.* (2018)<sup>[11]</sup> reported isolation of *Proteus spp.* from studies on prevalence of Gram negative bacilli in the intestinal tract of chicken.

*Citrobacter freundii* isolates were about 8.2 per cent of the total bacteria isolated in the study performed. In a similar study, Tawab *et al.* (2018)<sup>[13]</sup> identified that about 15 per cent of bacteria from *Citrobacter freundii* the intestine of chicken. The Gram-positive cocci isolated in the present study were *S. aureus* which constituted 6.9 per cent of the total isolates. In similar previous studies by Sengupta *et al.* (2011)<sup>[17]</sup>, Waters *et al.* (2011)<sup>[18]</sup> and Geidam *et al.* (2012)<sup>[19]</sup>, *S. aureus* were isolated from a substantial number of samples.

Geidam *et al.* (2012)<sup>[19]</sup> investigated the occurrence of multidrug-resistant bacteria in chickens. The most important antibiotics employed for the treatment of animals and humans were those belonging to beta-lactam antibiotics. Gram-negative bacteria mainly belonging to *Enterobacteriaceae* were having high potential of building up antibiotic resistance.

Carbapenems such as ertapenem, meropenem and imipenem were the most effective antibiotics used for the treatment of infection by the *Enterobacteriaceae* family. However, the resistance rate of the bacteria has increased in the present study. Apata (2009)<sup>[20]</sup> conducted a comprehensive investigation into the misuse to antibiotics in poultry leading to the prevalence of antibiotic resistance similar to that of present study.

All *E. coli* isolates were found to be completely resistant to CD and MET. Some isolates of *E. coli* were determined to be either resistant or intermediate sensitive to AMC, CTX, CIS, CIT, EX, GEN, NX, OF, IMP and MRP. Meanwhile, a few isolates of *E. coli* showed variability throughout the range in the case of CIT and ETP where it was observed that they were resistant, intermediate sensitive and susceptible. More or less similar findings were observed by Joshi *et al.* (2012)<sup>[22]</sup> and Hassan *et al.* (2014)<sup>[23]</sup>.

All *K. pneumoniae* isolates were found to be resistant to CTX, CD and MET. Some isolates *K. pneumoniae* were determined to be either resistant or intermediate sensitive to AMC, CIS, CIT, CIP, EX and GEN. Meanwhile, other isolates of *K. pneumoniae* showed a variability in the case of IMP. A similar result was recorded by Haeili *et al.* (2021)<sup>[24]</sup> and Tawab *et al.* (2022)<sup>[13]</sup>.

All *Salmonella paratyphi A* isolates were found to be completely resistant for CTX, CD, EX and MET. Some isolates of *Salmonella paratyphi A* were either resistant or intermediate sensitive to AMC, CIP and OF. Meanwhile, few isolates showed variability in the case of CIS, CIT, IMP, MRP and ETP. These results were similar to that recorded by Akbar and Anal (2013)<sup>[14]</sup>, and Hassan *et al.* (2014)<sup>[23]</sup>.

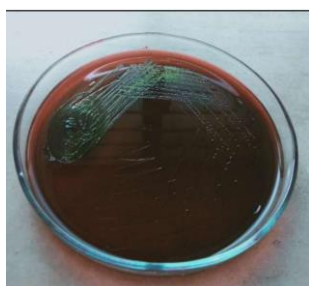
All *S. aureus* isolates obtained in the present study were found to be completely resistant to CTX Some *S. aureus* isolates were completely intermediate sensitive to NX and CIP. Few isolates were either resistant or intermediate sensitive to CD, IMP and MRP. Leftover isolates of *S. aureus* were found to be either sensitive or resistant to AMC, EX, OF and ETP. Further isolates were found to be either sensitive or intermediate sensitive to CIT, CIS and GEN. More or less similar observations has made by Sengupta *et al.* (2011)<sup>[17]</sup> and Waters *et al.* (2011)<sup>[18]</sup>.

The major organisms isolated from enteritis in chicken were generally *E. coli* and other *Enterobacteriaceae* like *Klebsiella spp.* and *Salmonella spp.* Isolates of *Staphylococcus aureus* and *Proteus mirabilis* were additionally found in some samples which were affected with enteritis. Most of the *E. coli* isolated showed a wide variety of resistance and intermediate resistance break points to most antibiotics of the penicillin class while being receptive to other classes.

**Table 1:** Shows the results from antimicrobial resistance of the isolates

Organism/ Antibiotics	<i>E. coli</i>	<i>K. Pneumoniae</i>	<i>Salmonella paratyphi A</i>	<i>Proteus spp.</i>	<i>Citrobacter spp.</i>	<i>S. aureus</i>
AMC	IM	IM	IM	S	IM	S
CIT	IM	IM	S	S	IM	S
CIS	IM	IM	S	S	R	S
CIP	S	IM	IM	S	R	S
CTX	IM	R	R	R	IM	R
CD	R	R	R	R	R	S
EX	IM	IM	R	S	R	S
ETP	S	S	S	R	IM	S
GEN	IM	IM	S	S	IM	S
IMP	IM	S	S	R	IM	S
MET	R	R	R	R	R	S
MRP	IM	S	S	R	R	S
NX	IM	S	S	R	R	S
OF	IM	S	IM	S	R	S

(Amoxicillin/clavulanic acid-AMC, ceftriaxone/tazobactam-CIT, ceftriaxone/subactum-CIS, ciprofloxacin-CIP, cefotaxime-CTX, clindamycin-CD, enrofloxacin-EX, ertapenem-ETP, gentamicin-GEN, imipenem-IMP, methicillin-MET, meropenem-MRP, norfloxacin-NX, ofloxacin-OF, resistance-R, intermediate-IM, sensitive-S).

**Fig 1:** *E. coli* on EMB**Fig 2:** *K. pneumoniae* on MCA**Fig 3:** *Salmonella paratyphi A* on XLD**Fig 4:** *Staphylococcus aureus* on blood agar

### Conclusion

The samples collected from enteric tract of 50 dead chicken died of enteritis brought to the post mortem facility in the Department of Veterinary Pathology, CVAS, Mannuthy reveals presence of 72 isolates out of which, 42 were identified as *E. coli*, eight as *K. pneumoniae*, three as *Salmonella paratyphi A*, 11 as *Proteus spp.*, three as *Citrobacter spp.* and five as *S. aureus* based on morphology, staining and biochemical tests.

Antibiotic susceptibility test revealed that most of the isolates exhibited high degree of resistance against antibiotics clindamycin and methicillin. Isolates showed either resistance or intermediate sensitivity against amoxicillin/clavulanic acid, ciprofloxacin, enrofloxacin, gentamicin, norfloxacin, ofloxacin. Isolates showed a variability in susceptibility in cases of ceftriaxone/subactum, ceftriaxone/tazobactam, cefotaxime, imipenem, meropenem and ertapenem. All isolates exhibited varying degree of drug resistance. Thus, the study could prove that antibiotic resistance built up significantly among the microbes in gastro intestinal tract of chicken, which could be a threat to human beings.

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