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Prevalence and Antimicrobial Resistance of *Staphylococcus aureus*, *Salmonella* and *Escherichia coli* Isolated from Poultry Meat in Tandojam, Hyderabad, Pakistan

Samia Sharafat[1], Dildar Hussain Kalhoro[1]*, Muhammad Saleem Kalhoro[1], Shahid Hussain Abro[1], Mazhar Hussain Mangi[1], Azhar Ali Laghari[2], Ali Raza Nizamani[3], Abdul Ahad Soomro[3], Rani Wagan[1], Asmatullah Kaka[1], Abdullah Channo[1], Hubdar Ali Kolachi[4], Muhammad Ibrahim Panhwar[4] and Mehkar Hussain[5]

[1]*Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam-70050, Pakistan*
[2]*School of Environmental Science and Engineering, Tianjin University, Tianjin 300350, China*
[3]*Central Veterinary Diagnostic Laboratory, Tandojam, Directorate of Veterinary Research and Diagnosis, Government of Sindh, Pakistan*
[4]*Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, 70060 Tandojam, Pakistan*
[5]*Department of Microbiology, University of Karachi, Karachi*

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SS, DHK, SHA and ARN conceived and designed the experiments. SS and DHK performed the experiments. MSK, MHM, AAS, AC, AK, HAK, MIP and MH analyzed the data. SS and DHK wrote the paper.

Key words
Antimicrobial resistance, Poultry, Meat, *Staphylococcus aureus, Salmonella, Escherichia coli*

ABSTRACT

Poultry meat is mostly consumed by people due to its good quality of protein and presence of all essential amino acids. In the present study, 100 poultry meat samples were randomly collected from district Hyderabad and cultured on selective media for the isolation and identification; mannitol salt agar for *Staphylococus aureus* (*Staph. aureus)*, Salmonella Shigella agar and brilliant green agar for *Salmonella* and MacConkey agar for *Escherichia coli (E. coli*.). Furthermore, biochemical tests were performed for the confirmation of bacterial species. Overall, 19%, 35% and 56% prevalence were found for *Staph. aureus*, *Salmonella* and *E. coli,* respectively. Antimicrobial resistance of fourteen antibiotics (gentamycin, erythromycin, penicillin, ampicillin, vancomycin, neomycin, tetracycline, oxytetracycline, doxycycline, bacitracin, cephalothin, norfloxacin, streptomycin and kanamycin) were performed by disk diffusion method against *Staph. aureus*, *Salmonella* and *E. coli* showed resistance against all drugs. However, *Staph. aureus* showed high resistance to norfloxacin 84%, penicillin and ampicillin 78%, tetracycline, oxytetracycline and kanamycin 73%, bacitracin, doxycycline and vancomycin 68%, cephalothin 63%, neomycin 36%, erythromycin and gentamycin 31% and streptomycin 15%. *Salmonella* showed high resistance to ampicillin 94%, penicillin 91%, oxytetracycline 88%, bacitracin and neomycin 85%, doxycycline and tetracycline 82%, erythromycin, gentamycin and cephalothin 80%, kanamycin 74%, vancomycin 68%, norfloxacin and streptomycin 62%. Whereas, *E coli* showed high resistance to penicillin and ampicillin 87%, norfloxacin 85%, tetracycline and doxycycline 82%, neomycin and streptomycin 80%, doxycycline 78%, vancomycin 76%, kanamycin 75% and gentamycin 8%. In conclusion, high prevalence of *Staph. aureus, Salmonella* and *E. coli* were observed in poultry meat. The bacteria were found to be highly resistant to penicillin and ampicillin. Proper use of antibiotics is recommended to control the emergence of drug resistance.

* Corresponding author: drdildarkalhoro@gmail.com
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INTRODUCTION

The unhygienic foodstuff is the major cause for the transmission of pathogenic microbes. In developing countries mostly, the mortality and morbidity occur due to the pathogenic bacteria (Gunasegaran *et al*., 2011). A high quality of protein source is fulfilled by poultry meat to the humans due to its sensorial characteristics.

Mostly the diseases occurred due to the consumption of unhygienic meat containing pathogenic bacteria meat such as *Salmonella* spp., and *Staphylococcus aureus* (European Food Safety Authority, 2009). The World Health Organization (WHO), and Food and Agriculture Organization (FAO) have reported high risk of *Salmonella* and C*ampylobacter* present in broiler meat (WHO, 1986, FAO, 2014). Chicken meat is contaminated by various microorganisms during slaughtering processing, environment and by handling equipment. Many of these bacteria are not destroyed during meat processing and storage. Consequently, bacterial infections are present in chicken meat which includes pathogenic species, causing severe gastrointestinal problems in humans (Authority, 2016). Chicken meat is one of the most well-known food worldwide as it contains numerous basic amino acids, minerals such as sodium, potassium, calcium, iron, phosphorous and vitamins like, B12 and niacin needed for life maintenance and development (FAO, 2014). Poultry meat is an ideal vehicle for bacterial development and known to hold countless microbes that are pathogenic to humans. This commonly happens in low disinfection levels, and may pose health concern to the customer if the meat isn't treated in a protected way (Zakaria, 2005).

Staph. aureus infections are associated with nosocomial infections (Kadariya *et al*., 2014). As a major foodborne pathogen it also causes the gastrointestinal disorders. In United States of America, total 241,000 foodborne cases were recorded per year caused by *Staph. aureus* (Scallan *et al*., 2011). Chicken meat is the key source of spreading staphylococcal foodborne diseases (Hanson *et al*., 2011). Generally, it has been reported in different countries that uncooked fresh and frozen chicken and turkey meat were contaminated by *Staph. aureus* (Waldroup, 1996). In China and Japan, raw chicken meat and their byproducts are being eaten by the people at high rate. Therefore, it is necessary to observe the actual contamination of enterotoxigenic *Staph. aureus* in raw chicken meat (Shimizu and Horie, 1999; Shimizu *et al*., 1991; Jiang *et al*., 2001). *Salmonella* species are causing foodborne diseases which has become major public health problem in Thailand. A high percentage of *Salmonella* infection was recorded in pigs, cattle, poultry and humans. Usually, *Salmonella* cause infections in humans through unhygienic food and mostly by consuming animal byproducts. The clinical signs of salmonellosis are nausea, vomiting, cramps, diarrhea and bacteremia may occur in severe cases. Thai food during a survey in 1993-1996 suggested that *S. enteritidis* and *S. anatum* were major contaminants found in frozen chicken and ready to eat meat (Boonmar *et al*., 1998). *S. enteritidis* found in chicken meat, caused salmonellosis in humans reported by Salmonella and Shigella Center of Thailand (Sakai and Chalermchaikit, 1996).

Escherichia coli is normally present in the intestinal microflora of animals and humans. *E coli* are beneficial bacteria but some strains cause diseases in humans, mammals and birds (Fairbrother and Gyeleka, 1994; Kaper *et al*., 2004). Pathogenic strains of *E. coli* are divided into two forms on the basis of producing intestinal and extra intestinal infection. Intestinal infection includes mild to severe diarrhea caused by enterotoxigenic or Enterohaemorrhagic *E. coli,* emerged from a hemolytic uremic syndrome (Kaper *et al*., 2004). Extra intestinal *E. coli* producing infection in both animals and humans like urinary tract infection, meningitis and septicemia. Uro pathogenic *E. coli* may become colonize into urinary tract hence, cystitis and polynephritis may occur which leads to urosepsis (Kaper *et al*., 2004).

Resistance of antimicrobial drugs has become serious health problem throughout the world. The resistance has occurred due to the misuse of drugs as a growth promoter in animals and birds (Normanno *et al*., 2007). The administration of antibiotics in food producing animals leads to bacterial resistance in humans via consuming food (Demirturk and Demirdal, 2004). More than 50 years antimicrobial drugs have been used in animal feed to enhance their activity and disease control. A prolonged usage of drugs in animal feed is considered as a major feature for drug resistance (Robredo *et al*., 2000; Lange and Brokking, 2005). Due to the consumption of raw chicken and turkey meat in most of the countries the resistant bacterial strains may transfer to the humans via food (Duman, 2007; Irlinger, 2008). The isolation of enterococci, *Staph. aureus*, Staphylococci and coagulase negative bacteria indicated high usage of antibiotics were used in chickens to control infections hence, the more antimicrobial resistance has occurred (De Boer *et al*., 2009; Kasimoglu *et al*., 2010). *Salmonella* infection may transfer to human from eating unhygienic meat (White *et al*., 2001). It has been recommended that resistant strains of *Salmonella* can cause serious diseases because of the presence of virulence factors (Verma *et al*., 2005).

The pharmaceutical industries of Germany, United Kingdom and France have made wonderful successes by introducing new antimicrobial drugs every three years. In the 21st century, antibiotic resistance was the most severe problem of the world. Few enteric human pathogens have become resistant due to the transmission of resistance genes or bacteria from animals to people through food chain (Barton, 2000). Food plays a significant role in the transmission of antibiotic resistance in terms of antibiotic residues or transfer of resistant genes from food microflora to pathogenic bacteria (Pereira *et al*., 2009). Present study

evaluated the presence of potential foodborne pathogens such as *Staph. aureus, Salmonella* and *E. coli* in poultry meat and furthermore to evaluate the antimicrobial resistance pattern of these pathogens.

MATERIALS AND METHODS

Collection of meat samples

One hundred fresh chicken meat samples were collected aseptically from local shops of Tandojam and District Hyderabad in polythene bags. The samples were transported in an ice box to the Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University and Central Veterinary Diagnostic Lab Tandojam and frozen at 4˚C till examined.

Antibiogram of bacterial isolates

To evaluate the susceptibility of isolated bacteria fourteen antibiotics of different groups were used, which include; gentamycin, oxytetracycline, doxycycline, kanamycin, tetracycline, ampicillin, streptomycin, norfloxacin, neomycin, penicillin, vancomycin, bacitracin, erythromycin, cephalothin. A pure culture colony was picked up and dispensed into the normal saline solution. The solution was mixed well to confirm that no dense material was evident. Clean swab was taken and immersed into the broth culture. Sterilized Mueller-Hinton agar plates were taken; swab was streaked on it and dried for 5 minutes. Seven different antibiotic discs were placed on the surface of the agar with the help of sterilized forceps. Cultured plates were overturned and incubated for 24 hours at 37° C. After incubation, inhibitory zones were measured by a metric ruler according to the Clinical and Laboratory Standards Institute (CLSI).

RESULTS AND DISCUSSION

Table I shows that out of a total of 100 meat samples from chicken meat were examined, 19 (19%) samples were found positive for *Staph. aureus*, 35 (35%) samples were found positive for *Salmonella* and 56 (56%) samples were found to be positive for *E. coli*. Similar results were recorded by Madhai *et al*. (2014) in Iran who recoded 6.42% prevalence in chicken meat products, while Akbar and Anal (2013) recorded 18.18% prevalence in Thailand. Pesavento *et al*. (2007) stated 28.6 % prevalence in Italy. Bhargava *et al*. (2011) recorded 25% prevalence in United States and 17.8% in Iowa. Hansona *et al*. (2011) recorded 42.1% prevalence in Oklahoma. Abdalrahman *et al*. (2015) also recorded 41% prevalence in USA. Our results are not in agreement with Gundogan *et al*. (2005) who reported 43.3% prevalence in Turkey. During present study 35 (35%) prevalence of *Salmonella* was observed. Similar investigation was done by Minami *et al*. (2010) recorded 25% of prevalence in chicken meat during the study in Thailand. Seza and Ayla (2010) reported 29.3% prevalence of *Salmonella* in poultry meat. Bundesinstitut für Risikobewertung (2009) reported 19.9% of prevalence of *Salmonella* from chicken meat. Bundesinstitut für Risikobewertung (2009) recorded the 17.4% prevalence of *Salmonella* in chicken meat. Boonmar *et al*. (1998) reported 72% of prevalence of *Salmonella* in chicken meat. Our results are not in agreement with Ellerbroek *et al*. (2010) who reported 13% prevalence of *Salmonella* in chicken carcass in Bhutan. During present study 56 (56%) prevalence was reported for *Escherichia coli* infection in poultry meat. These results are agreed to some extend with the study of Abd El Tawab (2014) who isolated *E. coli* at a percentage of 38%. Nehal (2009) and Mahajan (1994) reported the incidence of *E. coli* 41% to 60.9% in chickens.

Table I. Prevalence of *Staph. aureus, Salmonella* and *E. coli* isolated from poultry meat of Tandojam.

Bacterial isolates	No. of positive samples
Staphylococcus aureus	19 (19%)
Salmonella	35 (35%)
E. coli	56 (56%)

Table II show the efficacy of fourteen antibiotics against *Staph. aureus*, *Salmonella* and *E. coli*. *Staph. aureus* showed 15% resistance to streptomycin, 31% resistance to gentamycin and erythromycin, 36% resistance to neomycin, 63% resistance to cephalothin, 68% resistance to doxycycline, vancomycin and bacitracin, 73% resistance to oxytetracycline, tetracycline and kanamycin, 78% resistance to ampicillin and penicillin and 84% resistance to norfloxacin.

Salmonella showed 62% resistance against streptomycin and norfloxacin, 68% resistance to vancomycin, 74% resistance to kanamycin, 80% resistance to gentamycin, cephalothin and erythromycin, 82% resistance to tetracycline and doxycycline, 85% resistance to neomycin and bacitracin, 88% resistance to oxytetracycline, 91% resistance to penicillin and 94% resistance to ampicillin.

E. coli showed 8% resistance to gentamycin, 75% resistance to kanamycin, 76% resistance to vancomycin, 78% resistance to doxycycline, 80% resistance streptomycin, neomycin, bacitracin and erythromycin, 82% resistance to doxycycline and tetracycline, 85% resistance to norfloxacin, 87% resistance to penicillin and ampicillin.

Table II. Antimicrobial resistance of *Staph. aureus*, *Salmonella* and *E. coli* isolated from poultry meat from Tandojam.

Antimicrobial agent (µg)	*Staph. aureus* (n=19)	*Salmonella* (n=35)	*E. coli* (n=56)
Gentamycin (30)	6 (31%)	28 (80%)	5 (8%)
Oxytetracycline (30)	14 (73%)	31 (88%)	46 (82%)
Doxycycline (30)	13 (68%)	29 (82%)	44 (78%)
Kanamycin (30)	14 (73%)	26 (74%)	42 (75%)
Tetracycline (30)	14 (73%)	29 (82%)	46 (82%)
Ampicillin (10)	15 (78%)	33 (94%)	49 (87%)
Streptomycin (10)	3 (15%)	22 (62%)	45 (80%)
Norfloxacin (10)	16 (84%)	22 (62%)	48 (85%)
Neomycin (10)	7 (36%)	30 (85%)	45 (80%)
Cephalothin (30)	12 (63%)	28 (80%)	46 (82%)
Penicillin (10)	15 (78%)	32 (91%)	49 (87%)
Vancomycin (30)	13 (68%)	24 (68%)	43 (76%)
Bacitracin (10)	13 (68%)	30 (85%)	45 (80%)
Erythromycin (15)	6 (31%)	28 (80%)	45 (80%)

Staphylococcus was found highly resistant to norfloxacin (84%), penicillin, ampicillin (78%), oxytetracycline, kanamycin, oxytetracycline (73%), doxycycline, vancomycin, bacitracin (68%), cephalothin (63%), while mildly resistant to erythromycin, gentamycin (31%), neomycin (36%) and streptomycin (15%). Our results are in in agreement with other authors. *Staph. aureus* showed resistance to penicillin (Laxminarayan *et al*., 2013). Multi-drug resistance was detected in 22.2% of isolates, while maximum resistance was found against oxytetracycline (43.1%) and penicillin (23.8%). Oxytetracycline showed highest resistance among tested antibiotics in Thailand (Akbar and Anal, 2013).

*Salmonell*a was found highly resistant to ampicillin 94%, penicillin 91%, oxytetracycline 88%, neomycin 85%, doxycycline, tetracycline 82%, gentamycin, erythromycin, cephalothin 80%, kanamycin 74%, vancomycin 68% while mild resistance to streptomycin and norfloxacin 62%. Our results agree with Akbar and Anal (2013), who reported 73% resistance against tetracycline, 18.48% resistance to chloramphenicol, 36% to nalidixic acid and 27% resistant to ciprofloxacin. Odoch *et al*. (2017) reported 50% resistance to ciprofloxacin, 5.1% to tetracycline and 5.1% to chloramphenicol.

In our study *E. coli* was found highly resistant to ampicillin, penicillin 87%, norfloxacin 85%, oxytetracycline, tetracycline, cephalothin 82%, streptomycin, neomycin, bacitracin, erythromycin 80%, doxycycline 78%, vancomycin 76%, 75% kanamycin, while mildly resistant 8% to gentamycin. Our results are in agreement with other authors. Adeyanju and Ishola (2014) reported 4.9% prevalence of *E. coli* in Korea and 43% in Nigeria, while Joshi *et al*. (2019) 8.89% prevalence rate. Apun *et al*. (2008) isolated *E. coli* from broiler chicken in Malaysia and reported *E. coli* resistance to ampicillin, tetracycline and gentamicin with 11-95%. Rahman *et al*. (2008) recovered isolates of *E. coli* from poultry during his research work in Bangladesh and found *E. coli* resistance against chloramphenicol, ampicillin, ciprofloxacin, tetracycline and streptomycin in 37-87.5% cases; and 50-66.6% strains were highly sensitive to chloramphenicol and gentamicin 66-100%. While, Islam *et al*. (2008) reported *E. coli* resistance to tetracycline, penicillin, erythromycin and chloramphenicol. Tricia *et al*. (2006) reported 43% *E. coli* resistance against ampicillin.

CONCLUSION

Present study evaluated the prevalence of *Staph. aureus*, *E. coli* and *Salmonella* found in food chain. The chick meat samples showed 19% resistance to *Staph. aureus*, 35% to *Salmonella* and 56% *E. coli*. *Staph. aureus* was highly resistant to norfloxacin and penicillin, *Salmonella* was highly resistant to ampicillin and penicillin while *E. coli* was highly resistant to penicillin and ampicillin. In conclusion, *Staph. aureus, Salmonella* and *E. coli* are present as a foodborne pathogen in poultry meat. These pathogens are causing variety of diseases in humans via food chain.

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IRB approval

The research project was approved by the Directorate of Advanced Studies, Sindh Agriculture University, Tandojam, Pakistan.

Ethics statement

The study was conducted in line with the recommendations of Ethical Committee vide No. DAS 2674/ of 2019 of Director of Advanced.

Statement of conflict of interest

The authors have declared no conflict of interest.

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