The Antibiotic Susceptibility of the Isolated Bacteria from Chicken faces samples in District Mansehra, Region, KPK, Pakistan

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Abstract:

The poultry are exaggerated by enteric ailments; this ailment is one of the most important groups of diseases and is current to cause high financial losses in many areas around the globe due to increased death rates, decreased weight gain, increased medication costs and increased feed change rates. Several pathogens are involved as possible causes of enteric disorders either alone (mono-causal), in synergy with different other microorganisms. The objective of the study to determine the frequency of antibiotic susceptibility microbes in poultry industry. This study was conducted in district Mansehra region during the month of July to November 2018 to determine the frequency of antibiotic susceptibility microbes in the fresh chicken faces at different poultry farm of Dhodyal, Buffa and Shinkyari region of district Mansehra, Region, KPK, Pakistan. In this study total of 204 fresh faces sample were collected from poultry farm of district Mansehra region and then isolation and identification of the pathogenic bacteria were done by standard microbiological techniques. The number of 204 fresh faces samples of chicken were collected out of which 134 from Dhodyal, 40 from Baffa, and 30 from Shinkyari. The *E.coli*, *Salmonella*, *Shigella*, *Klebsiella*, *Proteus Pseudomonas*, *Staphylococcus* and *Streptococcus* were isolated from the tested. The antibiotic susceptibility pattern of the isolated bacteria was tested against 6 commonly used antibiotics Ampicillin, Enrofloxacin, Kanamycin, Cefotoxime and Gentamycin. *E. coli* shown a high resistance to Ampicillin followed by Streptomycin, while less resistance was noted against Gentamycin, Cefotoxime Kanamycin and Enrofloxacin. Salmonella show high resistance to Ampicillin followed by Streptomycin, Kanamycin, Cefotoxime, Enrofloxacin and Gentamycin, *Klebsiella*, *Shigella*, *Proteus*, *Pseudomonas Streptococcus* and *Staphylococcus* was shown high resistance against Ampicillin, followed by Streptomycin however less resistance was observed against Gentamycin Kanamycin Enrofloxacin and Cefotoxime. The present outcome gave a sign that poultry waste can fill in as a natural store for different anti-microbial resistant microorganisms and subsequently can fill in as potential courses for the passage of multidrug resistant zoonotic pathogen in the person. This has a very criticalness derivation for human wellbeing, as multidrug resistant diseases were risky to treat and every now and again required exorbitant antibiotics and long duration treatment.

Key Words: Chicken faces, Bacteria, Mansehra KPK Pakistan, Ampicillin, Enrofloxacin, Kanamycin, Cefotoxime and Gentamycin

INTRODUCTION:

Poultry sector play very essential role and fulfill the requirement of protein deficiency Poultry farms are used for raising chickens, turkeys, ducks, and other birds for egg or meat production.In Pakistan the commercial poultry sector was started in 1960. The government positive policy and the poultry farming community persistence was the result of initial growth of poultry industry (Sadiq, 2004). Although poultry are affected by enteric illnesses, this illness is one of the most important groups of diseases and is continuing to cause high economic losses in many areas around the globe due to increased death rates, decreased weight gain, increased medication costs and increased feed change rates. Several pathogens (Viruses, bacteria and parasites) are involved as possible causes of enteric disorders either alone (mono-causal), in synergy with different other microorganisms.

Poultry have been raised as backyard activities to fulfill the individual domestic desire. The department of poultry husbandry at the University of Agriculture Faisalabad was presented a better variety of chicken named Lyallpur Silver black in 1965-66. This breed was developed by crossing the native Desi breed with three imported breeds, namely New Hampshire White Cornish, and White Leghorn in a two-way cross breeding program to develop a breed that could Capable of producing up to 150 eggs per year and also have ability to survive under a harsh environmental condition of rural areas (Hussain et al., 2015)

In Karachi Sind province the first commercial hatchery was established in the collaboration of Pakistan International Air line with shaver poultry breeding farm of Canada at the mid of 1960 s, at the same time commercial poultry feed mill was built by liver brother private limited in district Rahim Yar khan, Punjab province (Memon, 2012). In 1970 the poultry sectors saw 20 to 30 percent growth per year and continued to grow at the rate of 10 to 15 percent in the 1980.The vibrant domestic marketing was the most significant reason for this growth due to which the chicken meat eating augmented more than 4 percent annually (Sadiq, 2004). One of the greatest and active part in Pakistan is the poultry production sector which contributing about 5.76 to 26.8 percent and 1.26 percent correspondingly to the total production of meat, cultivation sector, and overall GDP. In previous few years an excellent growth shown by the poultry industry and play a good role in the employment of peoples (Hussain et al., 2015). An overall growth of total birds, total chicken meat production and total number of eggs was experienced 177%, 297 and 271% respectively from 1917 to 1980 (Hussain et al., 2015).

The consumption of chicken meat is gradually increasing globally, the previous history and available information show that it reached to 14.2 kilogram per person, per annum (Rouger *et al.*, 2017). In 2010 the market share of mutton and beef had reduced to 20% to 55% while the poultry meat had increased to 25% respectively (Hussain *et al.*, 2015). The USA (united states of America) are the largest purchaser , with 49.8 kilogram per person per annum in all of the

developed western country.(Rouger *et al.*, 2017). The consumption of poultry meat increase is observed in the European Union and in countries of the Organization for Economic Co-operation and Development. Similarly, the eating of poultry meat in France, has double ended the previous 30 years and has become the second largest meat eating country since 2012 reaching more than 26 kilograms per person in 2014 (near to the utilization reported for the European union) after pork meat which is 32.5 kilogram per individual (Rouger *et al.*, 2017).Chicken carcasses cut and processed product are mostly eat amongst the poultry meat product (Rouger *et al.*, 2017).The poultry meat demand increasing primarily due to its receipt by most of peoples and its comparatively low contented of cholesterol. At present the poultry industry is facing a large number of environmental problems. One of the main problem for poultry industry is the accumulation of large amount of waste especially manure and litter (Power *et al.*, 2000; Kelleher *et al.*, 2002; Sharpley *et al.*, 2007).

The mixture of budding constituents, wasted feed, feather, and faces are collectively referred as chicken litter (Wilkinson et al., 2011; Kim et al., 2012).The faces of poultry are unwanted products defecated by poultry birds such as duck gees, turkey and chicken. Chicken faces can be in the form of water or semi-solid and its color contrasts between the species of birds. (Adegunloye, 2006). The composition of nutrient in the faces of poultry different with the type of birds, the amount of litter excrement, the manure handling system the feed allowanced, and the litter type. The chemical and physical composition of chicken faces can be vary and the composition affecting by factor include the amount of birds per unite area, the type of birds, the density of feed nutrient, environmental factor and other management factor (Adegunloye and Adejumo, 2014). Despite its economic advantage chicken litter contains a large and distinct population of microorganism .Microbial concentration in the chicken litter can reach up to 1010 CFU/G (Bolan et al., 2010)

Different microorganisms such as protozoan, fungi, algae, protests, archaea, and bacteria are the dynamic part of microbial diversity and these are usually briefly mentioned or not at all which requires consideration. Microorganisms are distributed throughout the biosphere. Bacteria like to live in an energy rich environment (Ganesan and Muthuchelian, 2009). The caeca of the gastrointestinal tract of poultry is one of the favorite places for bacteria which presented favorable environment for more than 200 bacterial strains. The species of Lactobacillus, Enterococcus and Enterobacteriaceae are the major micro flora of chick cecum at the first day of age. After second week of the chicks age, the species of Eubacteria and bacteroides species got established (van der Wielen *et al.*, 2001). Amongst the three regions of gastrointestinal tract of

chicken such as duodenum, cecum and ileum, duodenum has the lowest bacterial population while cecum has highest population of bacteria. The account of lactobacillus average is about $1x10^9$ in the ceca of chicken, along with lactobacillus huge amount of Enterococcus and Enterobacteriaceae were found. At the age of 12 day of chicks the account of facultative anaerobic and obligate anaerobic bacteria is 10 to 15 time more than aerobic bacteria.(Zhu *et al.*, 2002).

AIMS AND OBJECTIVES:

To determine The Antibiotic Susceptibility of the Isolated Bacteria from Chicken faces samples in District Mansehra, Region, KPK, Pakistan.

STUDY AREA:

This study was performed in the laboratory of Microbiology Department, Hazara University, and Mansehra. Different poultry farm of District Mansehra was vested for the collection of fresh feces samples from chicken.

METHODS AND MATERIALS:

SAMPLE COLLECTION:

A total 204 fresh chicken faces samples were collected from different poultry farms of District Mansehra region. The samples of fresh chicken fecal matter were taken randomly from chickens of different health status by using sterilized cotton swabs and transported to the laboratory.

TRANSPORT MEDIA:

Different transport media are used for delivering fecal samples to laboratories such as Campy-thioglycolate, Carry Blaire, Semi-solid motility test medium and alkaline peptone water. In the current research, sterilized cotton swab was used for transporting chicken fecal samples (without contamination and disruption of samples taken with culture swabs) to the laboratory 0f microbiology, Department of Microbiology, Hazara University within two hours for isolation and identification of different bacteria.

IDENTIFICATION OF MICROORGANISM:

The standard protocol of microbiology used for the identification of microorganisms.

SUCEPTIBILITY TEST:

The entire samples were inoculated on a specific media for microbial isolation like blood agar, Mac-Conkey agar and Salmonella, Shigella agar (S.S agar). The inoculated plates were being kept on incubation period at 37℃ for one day to two days. The isolation and identification of microbes were performing on the basis of morphological characteristic, culture and biochemical identification. The Antimicrobial susceptibility tests of isolated microbes were performed by disk diffusion method. Six different antibiotics were used which were given follow: Gentamycin, Cefuroxime, Kanamycin, Enrofloxacin, Streptomycin, Ampicillin.

In the present study, was conducted to investigate different pathogenic bacteria from chicken faces in three different regions of District Mansehra. Total samples of chicken faces were 204 that were collected from different poultry form. Out of these, 134 samples were collected from Dhodyal region, 40 samples from Baffa region and 30 samples were collected from Shinkyari region. All the samples were conformed primarily to the help of its growth characteristics on three different media such as Blood agar, MacConkey agar and Salmonella Shigella agar and then isolate were identified with the help of biochemical tests.

The isolated bacteria were further directed for checking the antibiotic susceptibility 6 commonly used antibiotics in veterinary practices. The antibiotic sensitivity was tasted by using the process of disc diffusion. The antibiotic includes Gentamycin, Cefotoxime, Kanamycin, Enrofloxacin, Streptomycin and Ampicillin. The zone of inhibition created by this antibiotic was measured by using scale. The result of these antibiotics was shown in following figures.

Antibiotic susceptibility profile of *E. coli*:

Antibiotic susceptibility profile of *E.coli* against various antibiotics showed that *E. coli* was most sensitive to Gentamycin (83.33%) followed by Cefotoxime (75.75%), Kanamycin (72.72%), and Enrofloxacin (68.18%) while the pathogen was most resistant against the antibiotics Streptomycin (90.90%) and Ampicillin (98.48%),) as shown in (fig 1.1)

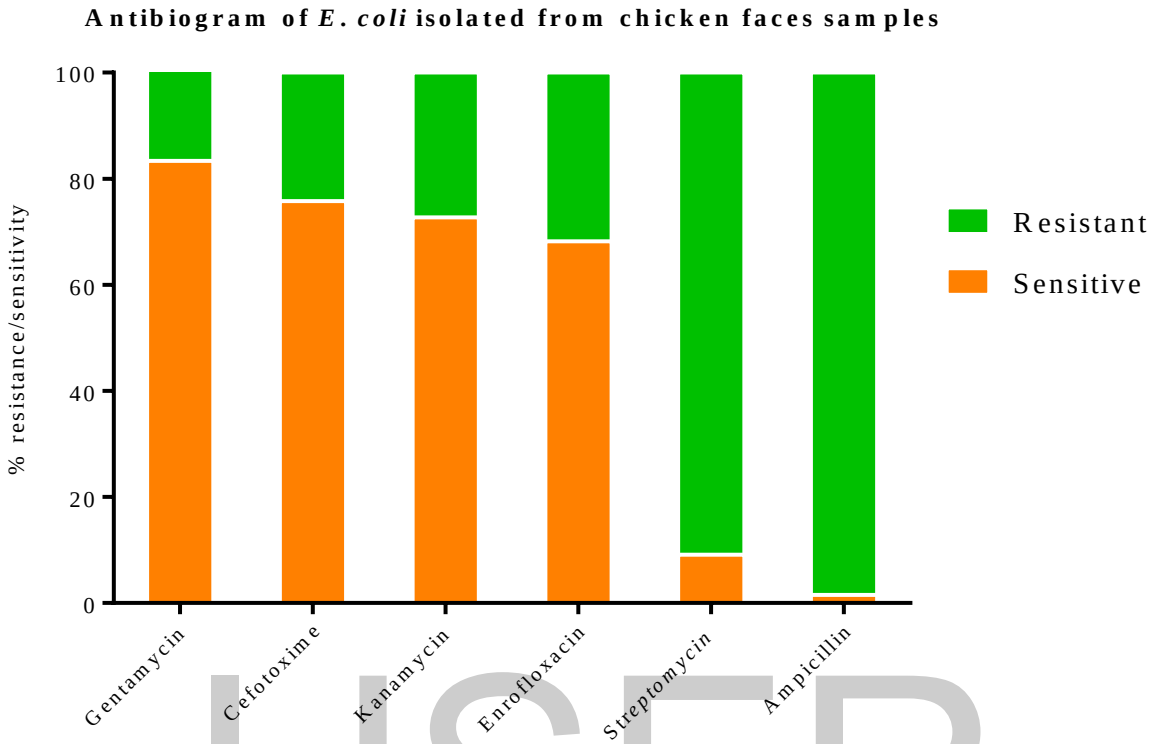

Figure 1.1: Antibiogram of *E. coli* for chicken faces in Dhodyal, Baffa and Shinkyari region. This figure shows the percentage of antibiotics susceptibility for these regions in District Mansehra.

Antibiotic susceptibility profile of *Salmonella*:

Antibiotic susceptibility profile of Salmonella against various antibiotics showed that Salmonella was most sensitive to Gentamycin (76.52. %) followed by Cefotoxime (65.38. %), Kanamycin (61.53%), and Enrofloxacin (69.23. %) while the pathogen was most resistant against the antibiotics Streptomycin (88.46%) and Ampicillin (96.15%),) as shown in (fig 1.2).

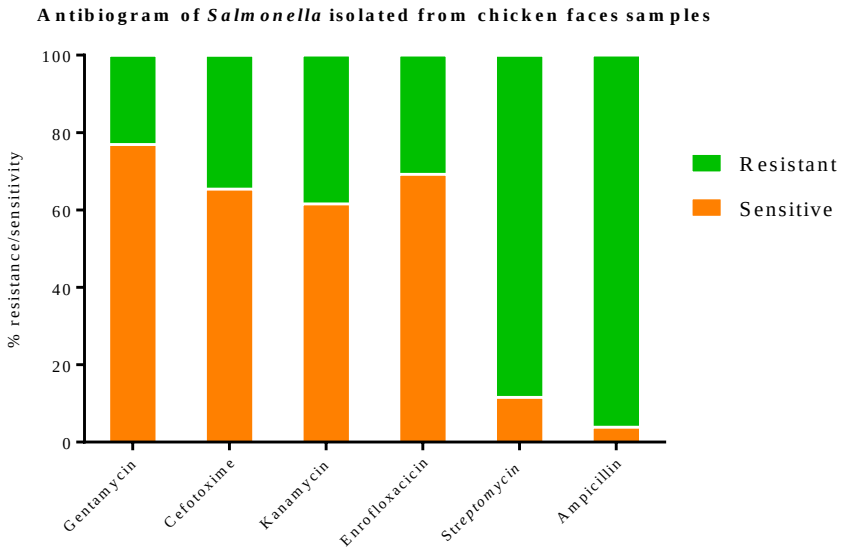

Figure1.2: Antibiogram of *Salmonella* for chicken faces in Dhodyal, Baffa and Shinkyari region. This figure shows the percentage of antibiotics susceptibility for these regions in District Mansehra

Antibiotic susceptibility profile of *Shigella*:

Antibiotic susceptibility profile of *Shigella* against different antibiotics showed that Shigella was most sensitive to Gentamycin (83.33 %) followed by Kanamycin (75. %), Cefotoxime (58.33%), and Enrofloxacin (66. 66%) while the pathogen was most resistant against the antibiotics Streptomycin (83.33%) and Ampicillin (100%),) as shown in (fig 1.3)

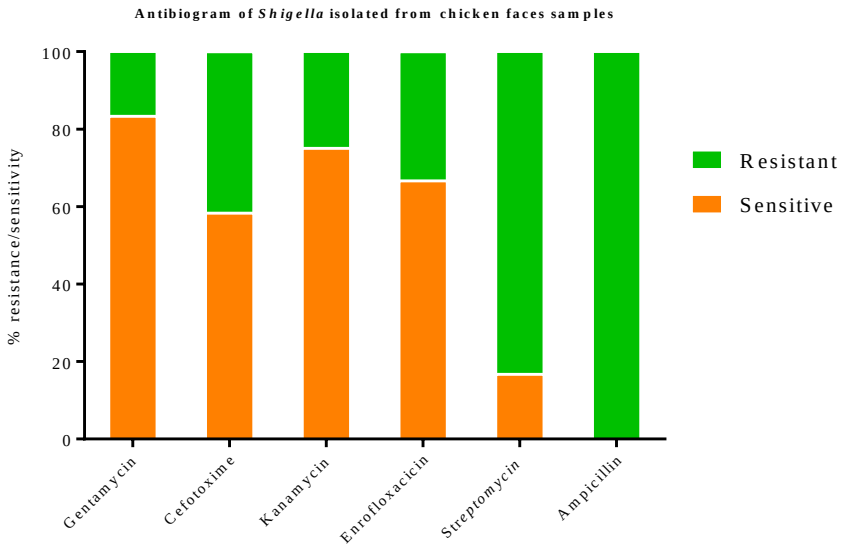

Figure1.4: Antibiogram of *Shigella* for chicken faces in Dhodyal, Baffa and Shinkyari region. This figure shows the percentage of antibiotics susceptibility for these regions in District Mansehra.

Antibiotic susceptibility profile of *Proteus*:

Antibiotic susceptibility profile of *Proteus* against different antibiotics showed that *Proteus* was most sensitive to Gentamycin (80 %) followed by %), Cefotoxime (60%), Kanamycin (80 %), and Enrofloxacin (60 %) while the pathogen was most resistant against the antibiotics Streptomycin (80%) and Ampicillin (100. %),) as shown in (fig 1.5)

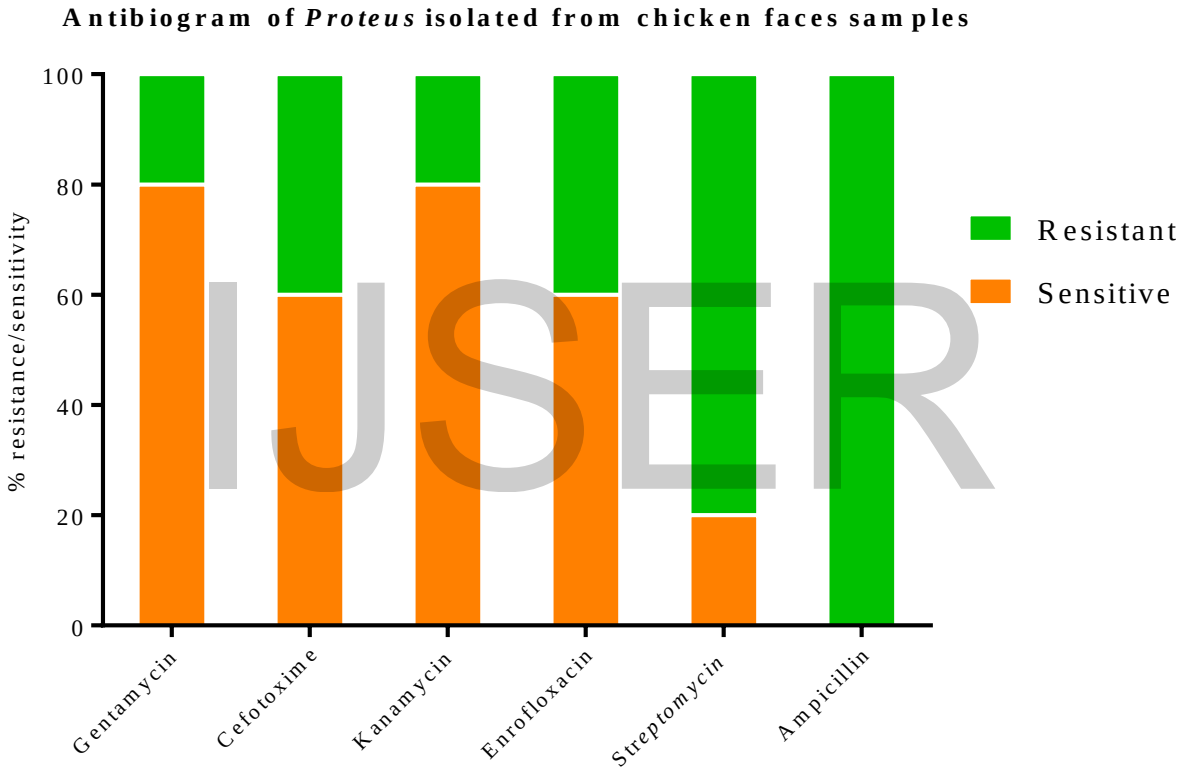

Figure1.5 Antibiogram of Proteus for chicken faces in Dhodyal, Baffa and Shinkyari region. This figure shows the percentage of antibiotics susceptibility for these regions in District Mansehra.

Antibiotic susceptibility profile of Pseudomonas:

Antibiotic susceptibility profile of *Pseudomonas* against various antibiotics showed that pseudomonas was most sensitive to Gentamycin (69.23 %) followed by %), Cefotoxime (53.86%), Kanamycin (46. %), and Enrofloxacin (46. %) while the pathogen was most resistant against the antibiotics Streptomycin (84.61 %) and Ampicillin (92 %),) as shown in (fig 1.6)

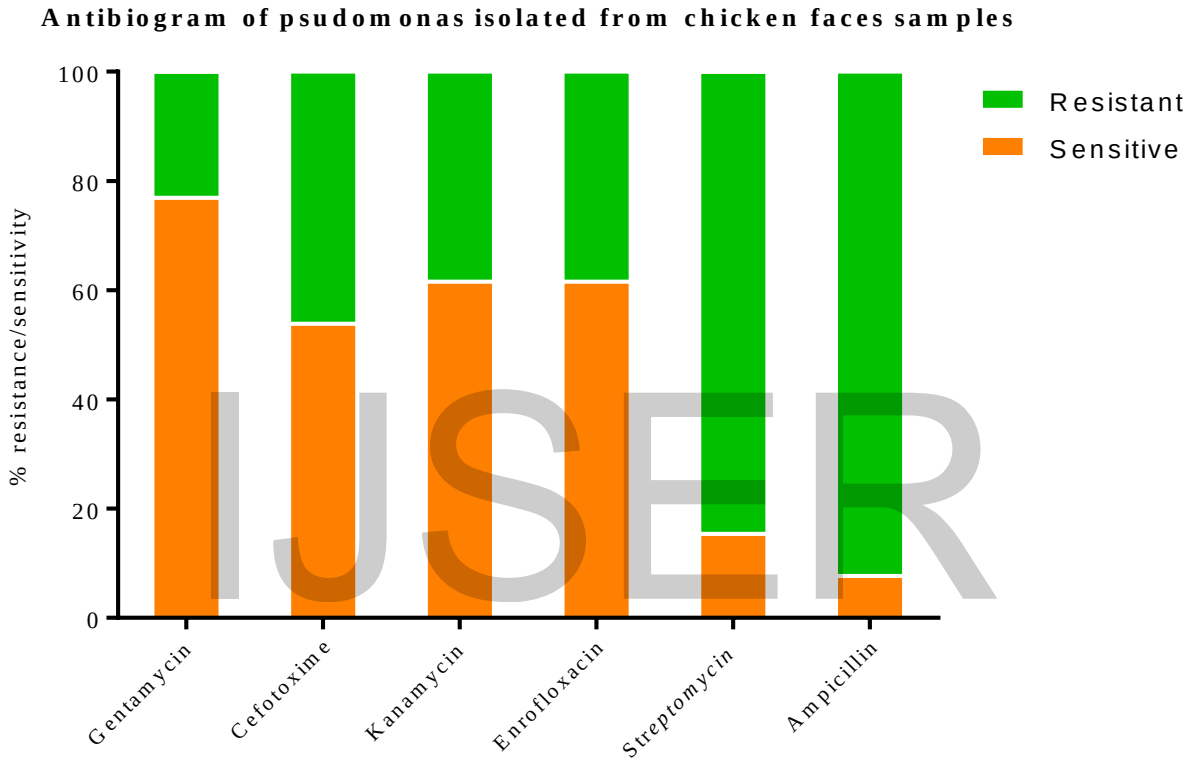

Figure1.6: Antibiogram of Pseudomonas for chicken faces in Dhodyal, Baffa and Shinkyari region. This figure shows the percentage of antibiotics susceptibility for these regions in District Mansehra.

Antibiotic susceptibility profile of Klebsiella:

Antibiotic susceptibility profile of *Klebsiella* against 6 different antibiotics showed that *Klebsiella* was most sensitive to Gentamycin (85.71 %) followed by Kanamycin (71. 42%), Enrofloxacin (71.42 %) and Cefotoxime (57.14%), while the pathogen was most resistant against the antibiotics Streptomycin (85.71 %) and Ampicillin (100%),) as shown in (fig 1.6)

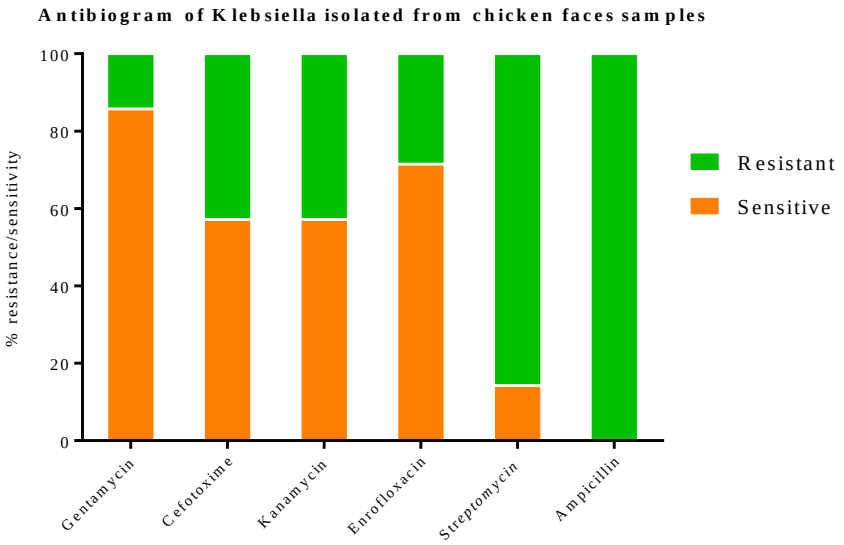

Figure1.6: Antibiogram of Klebsiella for chicken faces in Dhodyal, Baffa and Shinkyari region. This figure shows the percentage of antibiotics susceptibility for these regions in District Mansehra.

Antibiotic susceptibility profile of *Staphylococcus*:

Antibiotic susceptibility profile of *Staphylococcus* against 6 different antibiotics showed that *Staphylococcus* was most sensitive to Gentamycin (78.94 %) followed by%), Enrofloxacin (68.42 %), Kanamycin (63.15. %), and (55.63%), while the pathogen was most resistant against the antibiotics Streptomycin (89.47 %) and Ampicillin (94.73%),) as shown in (fig 1.7)

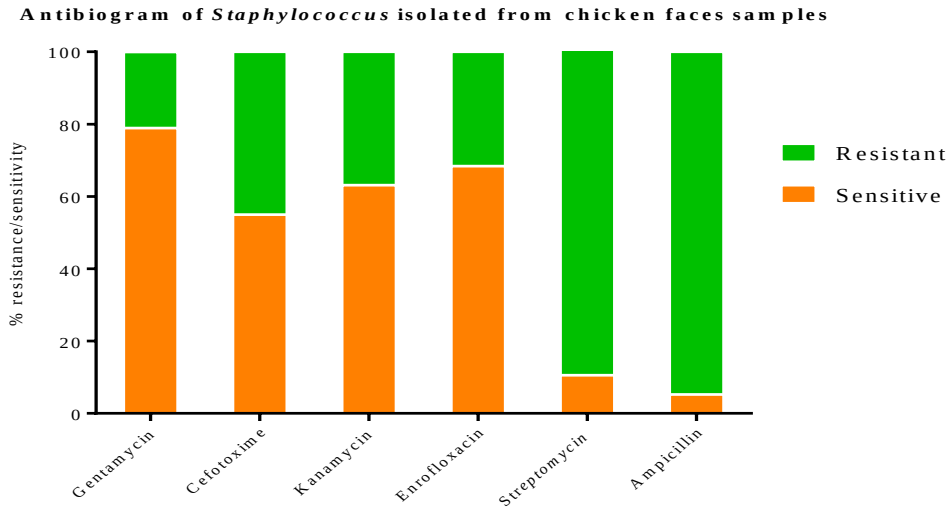

Figure1.7: Antibiogram of *Staphylococcus* for chicken faces in Dhodyal, Baffa and Shinkyari region. This figure shows the percentage of antibiotics susceptibility for these regions in District Mansehra.

Antibiotic susceptibility profile of *Streptococcus*:

Antibiotic susceptibility profile of *Streptococcus* against 6 different antibiotics showed that Streptococcus was most sensitive to Gentamycin (85.5 %) followed by%), Kanamycin (75%), , Enrofloxacin (75 %)and Cefotoxime (62.5%) while the pathogen was most resistant against the antibiotics Streptomycin (87.5 %) and Ampicillin (100%),) as shown in (fig 1.8)

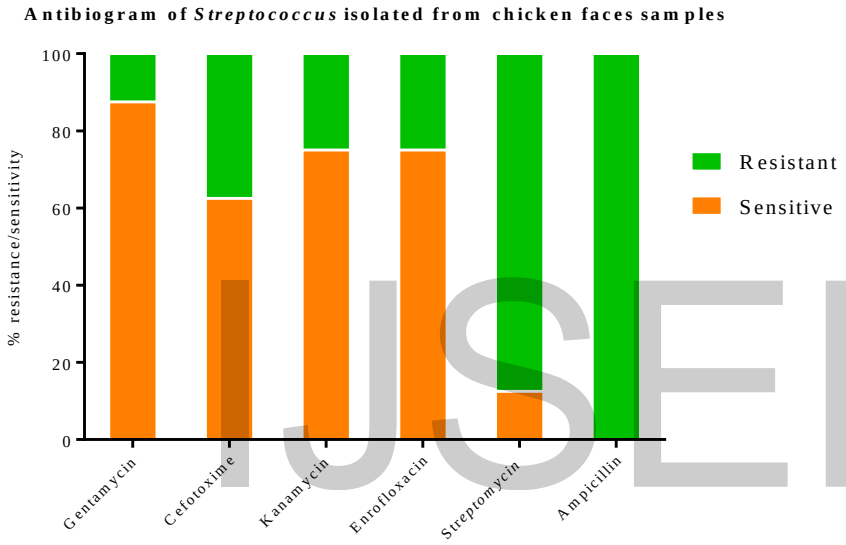

Figure1.8: Antibiogram of *Streptococcus* for chicken faces in Dhodyal, Baffa and Shinkyari region. This figure shows the percentage of antibiotics susceptibility for these regions in District Mansehra.

DISCUSSION:

In Pakistan poultry is an important agricultural industry and having an investment of more than 732 billion rupees. Poultry meat contained enough amount of protein having various important amino acids like methionine, histidine, leucine, cysteine, arginine, and isoleucine etc. (Uddin et al., 2018). Poultry meat is appreciated product for the consumer and their faces are used as fertilizer in District Mansehra region. The farmer used the chicken faces is good source of manure for the cultivation crops and vegetable. The use of poultry dropping for the cultivation of

crop serve the dual purpose elevating the soil for enhanced crops yield and economically disposing of the dropping. However, the directly addition of poultry dropping to field without any form of treatment possess some public health problem since they contain pathogenic microorganism. The pathogenic microorganism can contaminate the surrounding crops and vegetable and become the source of infection especially when such crops or vegetable are eaten row or brought home where they can contaminate other materials. The pathogenic microorganism can also be discharge onto surface that are used for drinking after run-off during rain fall. (Orji et al., 2005).

Pathogenic bacteria isolated in the present study included *E. coli, Salmonella, Shigella, Proteus, Staphylococci, Pseudomonas, Klebsiella, and Streptococci*. The *E. coli, Staphylococci, Salmonella and Pseudomonas* species were isolated from all three regions with highest prevalence in Shinkyari region compared to Dhodyal and Baffa. Contrarily, *Proteus, Streptococci* and *Klebsiella* species were only found in Dhodyal region and *Shigella* in Dhodyal and Baffa region of district Mansehra.

Furthermore, we have also performed antibiotic susceptibility testing of isolated bacteria. The antibiotics used in the present study included Gentamycin, Cefotoxime, kanamycin, Enrofloxacin, streptomycin, and ampicillin. Almost all the bacteria showed resistance to these antibiotics. *E. coli* isolates showed high resistance to Ampicillin (98.48.%.), followed by streptomycin 90.90%, Enrofloxacin 31.81%, Kanamycin 27.27%.and Cefotoxime 24% and Gentamycin 16.66%. In contrast to my study (Olukemi *et al.*, 2015) isolated *E.coli* from chicken carcass in Nigeria and showed 100% resistance to Ampicillin which is mostly similar to my Study. While (Ajayi and Omoya, 2017) isolated *E.coli* from chicken cloacal which showed 100% resistance to ampicillin, 71.4% resistance to streptomycin but high sensitivity to Enrofloxacin. Another study conducted in Zimbabwe, isolated *E. coli* from chicken with colibacillosis and shown a high resistance to Ampicillin 94.5% and 97% sensitive to gentamycin (Saidi *et al.*, 2012).

Salmonella isolated in the current study shown high resistance to Ampicilline,96%followed Streptomycin 86% Kanamycin 38.46%Cefotoxime 34.61% Enrofloxacin 30.76 and less resistance shown against gentamycin 23.06%.in distinction to my study (Mahmud, 2014) isolated Salmonella from egg and observed 100% resistance to beta lactam antibiotic (Ampicillin) and

less resistance was shown against Enrofloxacin 40%. A study was conducted in Nigeria in 2017 and isolated *Salmonella* from chicken and was shown a high resistance to Ampicillin,100% Ceftaxidime, 100% And were showed 100% sensitive to Enrofloxacin (Ejeh et al., 2017). The variation is due different sampling and antibiotic use.

Shigella isolated from the current study was shown high resistance to Ampicillin 100% followed. Streptomycin 83.33%, Cefotoxime 41.66% Enrofloxacin 33.33%, Kanamycin25% and Gentamycin16.66%.In contrast to my study (Rahimi *et al.*, 2017) isolated *Shigella* from meat and was shown high resistance to Gentamycin 100%,and ampicillin 84.61%, ciprofloxacin 38.46%.This variation is due to the sample type difference in our study I used chicken fecal simples where is other study used meat and meat product samples.

In the present study *Klebsiella* isolated from chicken faces and the rate of resistance to Ampicillin, 100% followed by Streptomycin 85%, Kanamycin 42.85%, Cefotoxime 42.85%, Enrofloxacin 28.75% and Gentamycin 14.28%. Observed in the present is lower than that observed in *Klebsiella* isolate from free rang chicken and shown a resistance to Ampicillin 90.9% Streptomycin 81.3% Enrofloxacin 90.9%. (Ojo *et al*., 2012) A study conducted in Dare Salam and isolated *Klebsiella* from chicken dropping and observed resistance to Ampicillin 46.4% Gentamycin 13.3% .(Mwambete and Stephen, 2015)

Staphylococcus isolated in the current research was showing high resistance to Ampicillin 94.73%followed by streptomycin 89.47%,Cefotoxime 45.%, Kanamycin,36.41% and Gentamycin 21.05%.while (Hanning *et al.*, 2012) isolated *S. aureus* from retailer chicken carcasses was showing high resistance to Ampicillin 100% and Gentamycin 11.7% which is lower from my study.

Proteus isolate in the present study shown high resistance to Ampicillin100%, followed by Streptomycin 80% Enrofloxacin 40%, Cefotoxime 40%, Kanamycin 20%, and Gentamycin 20% other study was conducted in Bangladesh in 2014 and isolated Proteus from poultry dropping and shown resistance to β-lactam antibiotic (ampicillin 66.6%, trimethoprim-sulfametxole, 66%, tetracycline 94.34%), amino glycoside (Gentamycin 53%), ciprofloxacin 16.75 %(Nahar *et al.*, 2014). Other study piloted in Nigeria 2016 and isolated Proteus from poultry farm rate showing 100% resistance to Ampicillin, Streptomycin, and 91% resistance to Kanamycin, 39%

levofloxacin. This variation from my study is due to different geographical variation, different diagnosis process.

In the present study *Pseudomonas* isolated from chicken faces was shown resistance to Ampicillin 92% followed by Stretomycin84.61% Kanamycin 53.84% Enrofloxacin53.84%, Cefotoxime 46%, and Gentamycin 30.76% while (Elsayed *et al.*, 2016) Isolated pseudomonas from poultry farm and shown a resistance to Amoxicillin 100% Ceftriaxone 80%. Cefuroxime 100% and Gentamycin sensitive. which is different from my finding, A study was conducted in Nigeria and isolated *Pseudomonas* from poultry product was shown almost resistance to all antibiotic (Iroha *et al.*, 2016). Study was conducted in Nepal 2017 and isolated *Salmonella Shigella ,Pseudomonas, E.coli, Proteus,* and *Klebsiella* from chicken meat and shown high resistance to Ampicillin 95% and less resistance to Gentamycin 16% (Shrestha *et al.*, 2017) which are similar to my finding.

In the current study *Streptococcus* isolated from chicken faces was shown resistance to Ampicillin 100% followed by Streptomycin 87.5%. Cefotoxime, 37.5% Enrofloxacin 25% Kanamycin 25%. and Gentamycin 12.5% in contrast to my study (Sharma *et al.*, 2017) isolated *streptococcus* from sick chicken throat air sac and trachea and show resistance to all selected antibiotic. A study was Conducted in Nigeria and isolated *Salmonella, Klebsiella, pseudomonas, Staphylococcus* from poultry litter and faces was shown 90% to 95 resistance to ampicillin resistance to Ampicillin, and 16 to Gentamycin % (Cookey and Otokunefor, 2016) which is related to my finding.

In short a number of risk factors can be there behind the high prevalence of bacteria in the present study. Some of the risk factors include direct exposure to outer environment in the summer season, contaminated water, contaminated feed, contaminated chicken faces, and humidity. These risk factors, in addition to cause high prevalence of bacteria, also results in the immunocompromising of normal chicken flock to opportunistic infections caused by *E. coli, Pseudomonas, Klebsiella* and *Streptococci*.

CONCLUSION:

The current result provided indication that poultry waste can serve as an environmental reservoir for multiple antibiotic resistance bacteria and hence can serve as possible routes for the entrance

of multidrug resistance zoonotic pathogen in the human being. This has very significance inference for human health, as multidrug resistance infections were problematic to treat and frequently required costly antibiotic and long term therapy. This can considerably raise the cost of treatment and even death. The study consequently endorsed appropriate information dissemination to agriculturalist and poultry feed maker about the public health prominence of proper poultry waste disposal.

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