Study the prevalence and Multidrug-resistant (MDR) of Salmonella enterica isolated from human and animals

Babar Hayat[1], Abu Baker Siddique[2], Sajjad ur Rahman[3*]

[1]Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan & Institute of Microbiology, Government College University, Faisalabad, Pakistan
hayatbabar200@gmail.com.

2. Institute of Microbiology, Government College University, Faisalabad, Pakistan.
absiddique@gcuf.edu.pk

3. Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan.
sajjadur@gmail.com **(Corresponding author)**

ABSTRACT

Salmonella enterica isolates from humans and nonhumans have antibiotic resistance patterns that are associated to diarrhea. Salmonella enterica infections are considered as a significant threat to humans in developed countries, with food animals being the primary source of infection. Multidrug-resistant (MDR) Salmonella are becoming more prevalent as a result of widespread antibiotic use in livestock and poultry production for a variety of objectives, including treatment and growth promotion, which has resulted in the evolution of antibiotic-resistant Salmonella, making treatment of Salmonella infections increasingly difficult with each passing year.

Salmonella was isolated and identified using enrichment media, selective media, and biochemical assays. Thirty-three (10%) of the 350 samples tested positive for human and domestic animal isolates. The majority of isolates with antibiotic resistance patterns were using antibiotics. Among isolated salmonella, the drug resistance patterns were Ciprofloxacin (45.5%), chloramphenicol (57.57%), ampicillin (66.66%), amoxicillin (39.33%), and oxytetracycline (51.51%). Human and poultry isolates had higher resistance than other isolates due to higher antibiotic use than other animals.

According to the findings, the prevalence of resistance isolates to ampicillin, oxytetracycline and is chloramphenicol is high, and their usage should be limited. Resistance to other antibiotics,

such as amoxicillin, and ciprofloxacin is also on the alarming level. This necessitates consideration in future infection control and antibiotic stewardship efforts.

This research is essential for one health since that reports potential public health pathogens connected with cattle and is important for studying antibiotic resistance patterns.

1-INTERODUCTION

There were 200,000 reported deaths globally from enteric fever in 2000, mostly in developing nations, with an estimated 22 million cases. The incidence and fatality rate of enteric fever vary by region, however in the absence of antibiotic therapy, the mortality rate might reach 7%. (Crump et al. 2004).

Enteric fever is endemic in many parts of the African and Asian continents, as well as in countries such as Europe, South and Central America, and the Middle East. The total number of Salmonella cases in the United States and certain European countries is less than 10 per 100,000 people each year. The disease was introduced by immigrants or travelers returning from Africa, India, or Pakistan in the majority of cases reported in these nations. (Molbak *et al*.,2002).

Many Asian countries, including China, India, Vietnam, Pakistan, and Indonesia, have high rates of enteric fever, with more than 100 cases per 100,000 people per year. Pakistan and India have the highest incidence rates in Asia, with 451.7 and 214.2 incidents per 100,000 population, respectively (Ochiai *et al*., 2008).

Enteric fever is a growing problem in southeast Turkey, where 10,000 people are identified each year (Iseri et al., 2009). The major issue with this enteric fever is antibiotic resistance, which aids in the development of new antibacterial medications. Salmonella is resistant to medicines such as ceftriaxone, cotrimoxazole, and ampicillin, yet effective medications such as ciprofloxacin are available. Salmonella typhi, a multidrug resistant (MDR) strain, is common and is the source of many endemic and epidemic typhoid fever illnesses. The MDR strain of S. enterica is resistant to current drugs, resulting in a high death rate and a high potential for spread. There are some that are difficult to manage (Sonja *et al*., 2015).

Salmonella spp. can spread through a wide range of foods, including meat, eggs, chicken products, and milk. Consuming these items after they have been contaminated with Salmonella can cause infection, including gastroenteritis and typhoid fever, which accounts for 95% of cases of salmonellosis in humans (Jajere, S.M. 2019).

Salmonellosis is a zoonotic bacterial disease that is primarily spread to people through tainted food and water. Salmonella species are most commonly transmitted to humans through poultry items and raw meat. In fact, the slaughtering process of food animals such as poultry and cattle is considered as one of the most important routes of Salmonella transmission to human. Salmonellosis affects the gut, and the most common symptoms of infection are abdominal cramps, fever, vomiting, and diarrhea (Gut *et al*., 2018).

The widespread use of antibiotics in cattle and poultry farming for a number of purposes, such as prophylaxis, growth promotion, and treatment, has led to the emergence of antibiotic resistant serotypes, making the management of Salmonella infections more challenging with each passing year (Rahmani *et al*., 2013).

In mid 1970s chloramphenicol was the unknown drug for the treatment of this fever and mortality rate reduced 10% to greater than 2% (Woodward *et al.*, 1948).

Salmonella Typhi resistance to tetracyclines, ampicillin, streptomycin and additional resistance to streptomycin and sulphonamides reported in Pakistan and India. This variability due to multidrug resistance (MDR). Chloramphenicol-resistant strain of *Salmonella Typhi* informed in many countries but a strain has plasmid-resistance chloramphenicol. Immunobiological properties, immunochemical and biochemical properties of chloramphenicol resistance of *S. Typhi* and chloramphenicol sensitive strains, no found in chemical composition of two strains. Immunological investigation expressed that chloramphenicol resistance strain contain less the *Vi* antigen, it was less virulent in mice and active and passive mouse ability lower than that chloramphenicol. The MDR strain of S. enterica is treatment resistant, resulting in a high death rate and the potential for dissemination. Some are more difficult to manage than others (Sonja *et al*., 2015).

S.enterica isolates from Algeria with multidrug resistance were isolated from stool and blood samples. He investigated agar dilution with IPA. In this study, the 6,178 strains tested positive

for 96.15%, 1.86% for 115 *S.paratyphi*, and 1.99% for 123 *S. paratyphi* A. There was no resistance found in the seven strains examined. (Mirza et al., 1996) investigated between January and July of 1994. He chose 692 feverish patients from Quetta Military Hospital in Baluchistan (Pak). He isolated Salmonella spp. from 76 different patients. *S.enterica* was isolated from 62 individuals, and *S.paratyphi* was isolated from 14 patients. *Salmonella Typhi* was detected during the hot, dry months of May and June, followed by February and March. *Salmonella Typhi* isolates with multidrug resistance were detected in 43 (69%) of them. This resistance was not investigated in S. paratyphi.

2-METHODOLOGY

2.1 Samples collection

350 samples were collected from human and animal cases that represented the signs and symptoms of diarrhea and fever. Every sample was collected in a sterilized container and placed at 4^0C.

2.2 Isolation

All faecal samples were transferred to Tetrathionate broth and cultured for 48 hours at 37^0C. The tetrathionate broth was produced according to the recipe, autoclaved at 121^0C for 20 minutes, and fortified with iodine. Each sample (2 gm homogenised) was inoculated into tetrathionate broth, and the growth was subcultured on Salmonella Shegilla agar (SSA) after 48 hours at 37^0C incubation.

2.3 Identification of isolates

Gram's staining, triple sugar iron test, indole test, catalase test, methyl red test, Voges-Proskauer test and citrate utilization test were used to identify isolates morphologically.

2.4 Antibiotic Susceptibility testing

The Kirby-Bauer technique on Mueller Hinton Agar was used to determine antibiotic sensitivity in accordance with CLSI standards. Isolated salmonella from respected specimens were dispersed on the respected plate, then five antibiotic sensitivity discs were placed using sterile forceps and incubated for 24 hours at 37^0C. Antibiotic sensitivity tests were performed using

antibiotic discs containing Ciprofloxacin (10g), Chloramphenicol (10g), Ampicillin (10g), Amoxicillin (10g), and Oxytetracycline (10g). The plates were incubated at 37^0C for 24 hours to test their resistance to antibiotics.

3- Results

3.1 From 65 diarrheal patients collected at the DHQ (District Head Quarter Hospital) in Faisalabad. Pakistan, four (6%) isolates were positive. The patient's history was reported. Six (10%) isolates were positive from 60 Poultry droppings samples taken at a poultry farm (UAF). Seven (12%) infections were positive from 55 cow faecal samples from Livestock farm (UAF), and ten (11%) isolates were positive from 110 cat and dog faecal samples from CMS.UAF. Six isolates were found to be positive in 60 sheep faeces collected from a livestock farm (UAF).

On SSA, all isolates produce black-centered colonies. This dark precipitate on SSA is caused by H2S produced by sodium thiosulphate. Similar number of isolates were positive on XLD (Xylose Lysine Deoxycholate) agar.

Gram's staining was used to study morphological identification. These were Gram-negative and coccobacillus bacteria.

All isolates tested positive for TSI. Shows a specialized test for *Salmonella enterica* the extremely little percentage of TSI media dark due to H2S formation, no gas production, and acidic butt after 48 hours incubation at 37^0C.

The results of all biochemical and fermentation tests performed on these isolates. All isolates were TSI positive. In the instance of VP, all isolates were negative (100%). All isolates tested positive for methyl red, 81% tested positive for indole, 81% tested positive for citrate, and 81% tested positive for catalase. Salmonella motility testing revealed that all isolates from a total sample were motile.

Antibiotic Susceptibility testing

Table 3 (A) represented the results of antibiotic susceptibility test from different isolates. Isolates of Salmonella *enterica* from human source were resistance to chloramphenicol/ ampicillin

/amoxicillin/oxytetracycline and sensitive to ciprofloxacin. Salmonella isolated from poultry showed resistance to chloramphenicol/ ampicillin/ oxytetracycline and sensitive to ciprofloxacin and amoxicillin. Salmonella isolated from horse were resistance to ciprofloxacin/chloramphenicol/ oxytetracycline. *Salmonella* isolated from cat were resistance to chloramphenicol/oxytetracycline. In the cause of dog were resistance to Ampicillin / oxytetracycline. Salmonella isolated from Cattle were resistant to oxytetracycline.

Table:3 (A) Antibiotic Sensitivity and Resistance Patterns of Isolated Salmonella

fecal samples	Sample size	Ciprofloxacin 16-20mm/10µg		Chloramphenicol 20-21mm/10µg		Ampicillin 14-15mm/10µg		Amoxicillin 14-15mm/10µg		Oxytetracycline 14-15mm/10µg	
		R S		R S		R S		R S		R S	
Poultry	5	2(15)	3 (23)	4 (18)	1(23)	4 (13)	1(17)	1(12)	4(17)	3(12)	2(16)
Cow	5	1(15)	4 (22)	2(18)	3(23)	2(12)	3 (17)	1(13)	4(19)	3(11)	2(17)
Sheep	5	2(14)	3 (21)	1(19)	4 (22)	4(13)	1(16)	2(13)	3 (16)	1(13)	4(17)
Cat	4	1(13)	3 (22)	3 (19)	1(23)	2(13)	2 (16)	1(14)	3 (17)	3(11)	1(17)
Dog	5	2(13)	3 (22)	3(19)	2 (22)	4(14)	1(15)	1(13)	4 (16	Inter	(14.5)
Human	6	5(15)	1 (22)	4 (17)	2(22)	4 (12)	2(16)	5 (11)	1(16)	5(13)	1(16)

Table: 3 (B) Percentage of Antibiotic Sensitivity and Resistance Patterns of Isolated Salmonellae

fecal samples	Sample size	Ciprofloxacin 16-20mm/10µg		Chloramphenicol 20-21mm/10µg		Ampicillin 14-15mm/10µg		Amoxicillin 14-15mm/10µg		Oxytetracycline 14-15mm/10µg	
		R % S%		R% S%		R % S%		R % S%		R % S%	
Poultry	5	2(40%)	3(60%)	4 (80%)	1(20%)	4(80%)	1(20%)	1(20%)	4(80%)	3(60%)	2(40%)
Cow	5	1(20%)	4(80%)	2(40%)	3(60%)	2(40%)	3 (60%)	1(20%)	4(80%)	3(60%)	2(40%)
Sheep	5	2(40%)	3(60%)	1(20%)	4 (80%)	4(80%)	1(20%)	2(40%)	3 (60%)	1(20%)	4(80%)
Cat	4	1(20%)	3(80%)	3 (80%)	1(20%)	2(50%)	2 (50%)	1(20%)	3 (80%)	3(80%)	1(20%)
Dog	5	2(40%)	3(60%)	3(60%)	2 (40%)	4(80%)	1(20%)	1(20%)	4 (80%)	Inter	(14.5)
Human	6	5(80%)	1(20%)	4 (80%)	2(20%)	4(66%)	2(33%)	5 (80%)	1(20%)	5(80%)	1(20%)

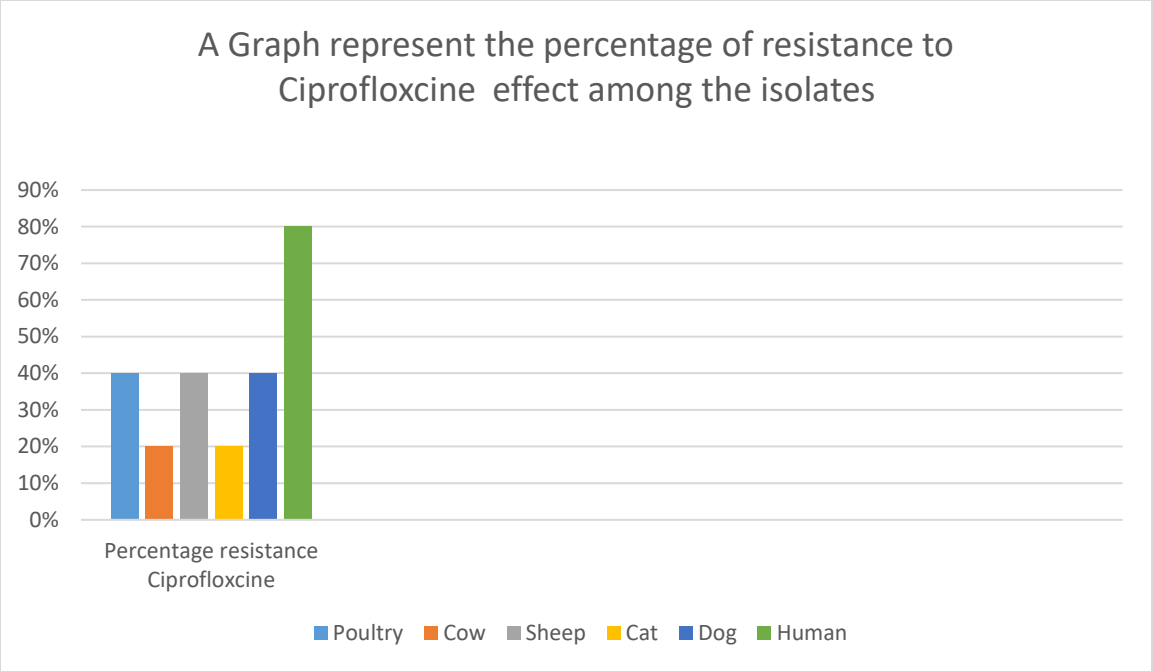

Figure 01: This study looked at the origin and geographical dynamics of 350 isolates of Salmonella from Humans, animals, foods originating animals, and the environment were among the several sample sources. 350 isolates' prevalence, geographic distribution, and several sources were discovered. In the case of ciprofloxacin antibiotics, the above graphs show a higher percentage of antibiotic resistance in human isolates as compared to isolates from other sources.

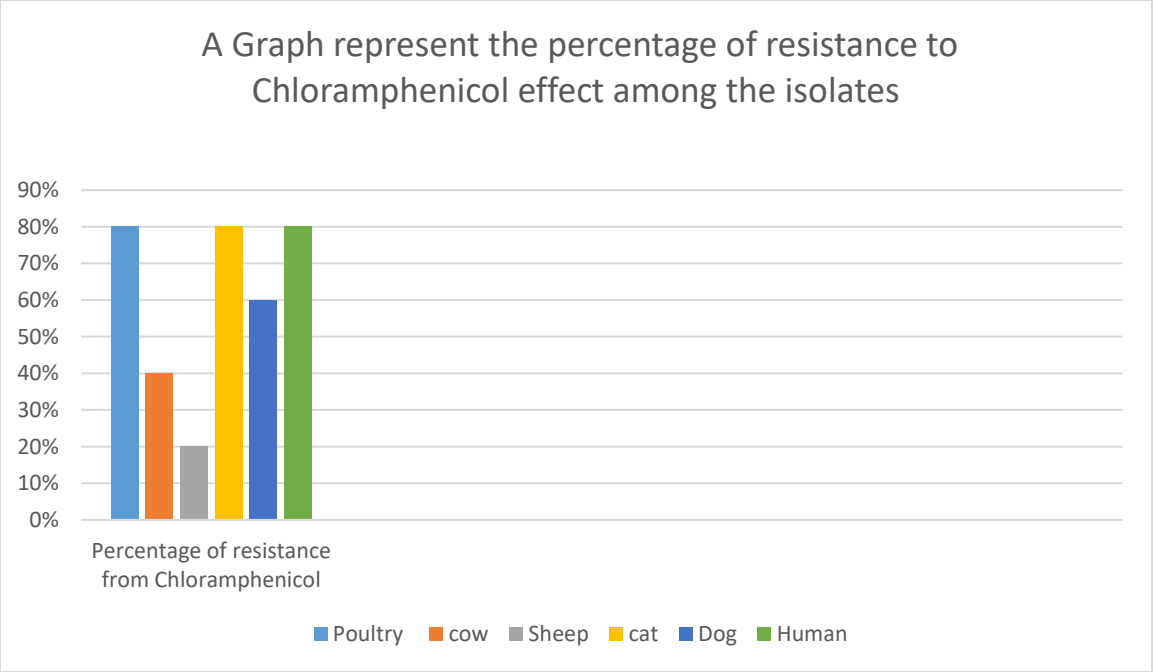

Figure:02 The figures above illustrate the same proportion of chloramphenicol antibiotic resistance in isolates from humans, poultry and cats antibiotics as compare to isolates from other sources.

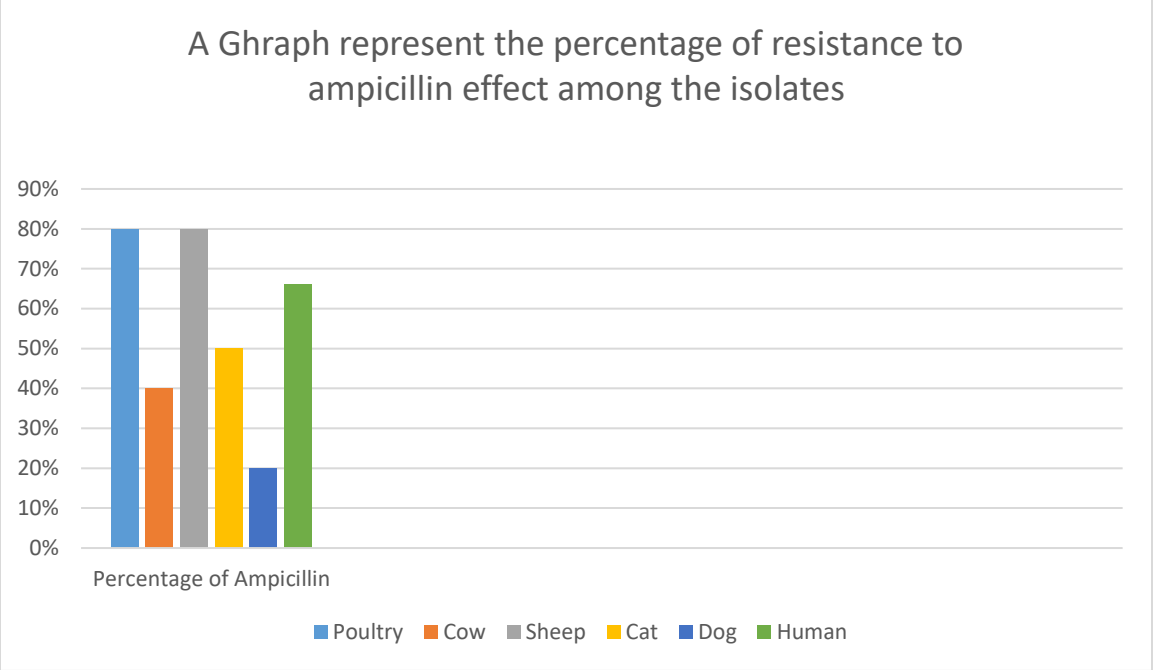

Figure:03 According to the figures above, human, poultry, dog, and sheep isolates had a higher percentage of antibiotic resistance ampicillin to than isolates from other sources.

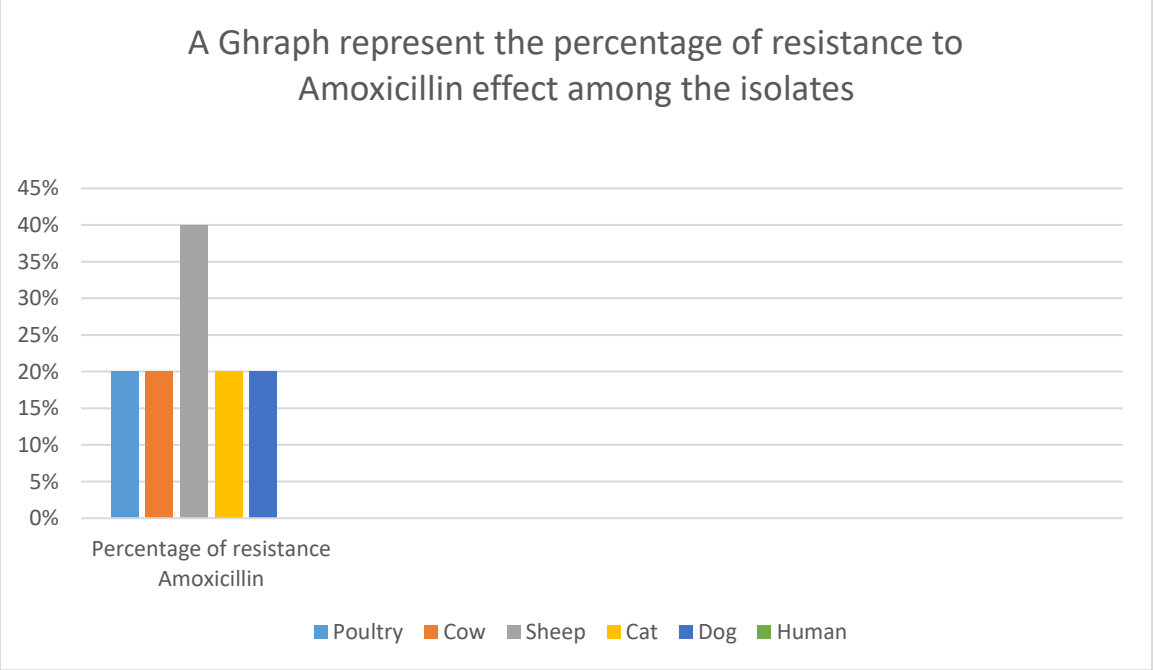

Figure:04 The figures above show that human isolates have a higher percentage of antibiotic resistance to amoxicillin than isolates from other sources**.**

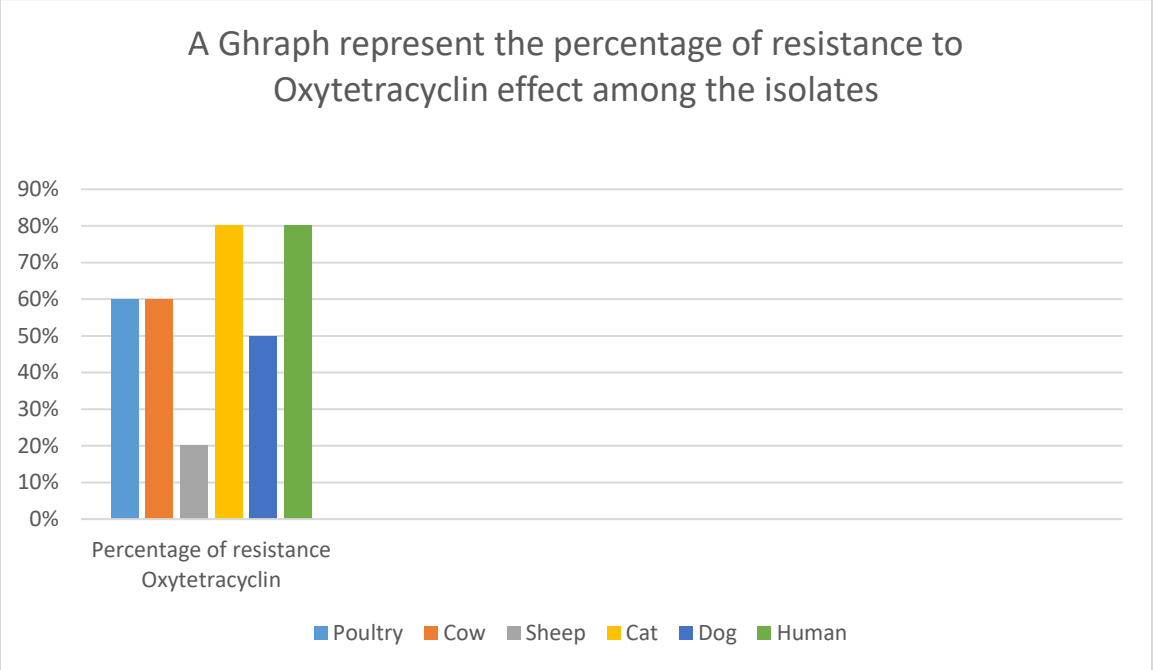

Figure: 05 The above-mentioned statistics show that oxytetracycline antibiotic resistance was more prevalent in human, poultry, and cat isolates than in isolates from cows, sheep, or poultry.

The graph represents the MDR profiles of Salmonella enterica isolates from human and animals including antibiotic resistance to Ciprofloxacin (16-20mm/10g), Chloramphenicol (20-21mm/10g), Ampicillin (14-15mm/10g), Amoxicillin (14-15mm/10g), and Oxytetracycline (14-15mm/10g) and also shows a comparison of antibiotic resistance among Salmonella enterica isolates from different sources.

Isolates were stored in 15ml tubes with 3ml of BHI broth at 180rpm for 4-6 hours at 37 °C in a shaking incubator to establish exponential growth phase.

4- Discussion

This study discovered an antimicrobial resistance trend in Salmonella isolated strains from animal and human gastrointestinal sources. Worldwide, human salmonellosis is regarded as a major public health risk. Salmonella isolates from humans, poultry, and horses showed increased

resistance or decreased susceptibility to certain antimicrobial agents. The final purpose of this research was to investigate the trend of antibiotic resistance. Human and poultry salmonella antimicrobial resistance patterns were found to be connected because humans consume poultry and Salmonella from poultry is transferred to humans. In a study of multidrug resistance patterns in humans, poultry, and horses, nearly 80% were resistant to ciprofloxacin, chloramphenicol, penicillin, amoxicillin, and oxytetracycline. Increased drug resistance implies the usage of explosive medicines in medication. These medications are simple to use and are highly recommended by doctors. The prescription and sale of drugs are governed by laws. Pharmacists are not required to follow drug prescription and sale restrictions.

In the case of ciprofloxacin Isolated salmonella from poultry, cow, sheep, cat, dog, human, and horse were resistant to chloramphenicol in the current study, with 60-80% resistance in poultry, cat, dog, horse, and human. In the case of poultry, humans and horses are 20% sensitive to chloramphenicol, whereas sheep and cows are 80% and 60% sensitive, respectively. The antibiotics used in growth boosting agents are the cause of the high resistance pattern found in isolates associated with cattle. Salmonella resistance to chloramphenicol was higher in poultry and humans than in sheep and cows. The findings are consistent to previous research that indicated 80-90% (Hatta 2008) and 100% (Hatta 2010). (Misra 2005).

To protect public health from illnesses caused by multidrug-resistant Salmonella spp., novel research into bacteriophage therapy, the development of alternative medicines, and the rapid detection of pathogens are required. Our findings indicate that there are knowledge gaps in Pakistan's present safety and hygiene practices. As a result, we suggest that appropriate strategies be implemented to reduce the prevalence of Salmonella spp. in the food chain in order to protect public health (Hossain *et al*., 2022).

5-Conclusion

Our investigation revealed that Salmonella isolates recovered from various sources, including humans in Pakistan, had a significant frequency of antibiotic resistance. Our findings offer the first overview of the increasing treatment with antibiotics results resistance of Salmonella, which is responsible for human cases of salmonellosis in Pakistan. This information is important for public health and food safety. The outcomes we have here are more typical of Pakistan and may prove helpful in the future when assessing potential risks.

Consequently, epidemiological studies on Salmonella infections in humans and animals must be ongoing, and additional research is required to improve our knowledge of the emergence and spread of MDR strains**.**

One of the main sources of Salmonella is poultry products, and it's possible that the bacteria's increased multidrug resistance will lead to an increase in human infections.

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