ISSN (Online) = 2522-6754 ISSN (Print) = 2522-6746
WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY
Research Manuscript www.sciplatform.com

Analysis of bacterial diversity associated with commercial broiler chicken in Lahore, Pakistan

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Authors' Contribution	**Azam, F.** conducted the experimental work and collected the data; **S. Tanveer** prepared the draft of the manuscript; and **B. Ali** conceived this study, performed the statistical analysis
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Review Proccess: peer review	
Digital Object Identifier (DOI) Number:	https://dx.doi.org/10.33865/wjb.007.02.0540

ABSTRACT

Chicken (*Gallus gallus domesticus*) is the most widely domesticated animal worldwide. Food biosafety is a great concern for consumers, producers, and public health organizations around the world. The poultry sector contributes 3.1% to the GDP of Pakistan. Infections caused by bacteria and viruses in poultry pose a serious threat to consumer health and economic burden in Pakistan while accurate figures are still unknown due to several factors, including inadequate surveillance, management, monitoring, and infection control. The chicken samples were procured from Shadman, Moon, Karim Block, Tollinten, Jallo and Wapda Town meat market of Lahore, Pakistan. Overall, 63 bacterial strains were isolated from complex and selective media and further processed for characterization. The 16S rRNA gene sequencing of bacterial strains were exhibiting genetic similarity with *Proteus, Shigella, Providencia, Escherichia, Citrobacter, Klebsiella, Myroides, Enterococcus, Serratia, Staphylococcus, Bacillus, Macrococcus* and *Kluyvera*. Bacterial strains were evaluated for antibiotic susceptibility testing, swarming motility, and biochemical tests. Antibiotic susceptibility was tested by using discs of tetracycline, gentamycin, streptomycin and chloramphenicol. Bacterial strains were recorded as resistant to all antibiotics except chloramphenicol. It was confirmed that chicken meat was infested with potential opportunistic human pathogens. To overcome the foodborne illnesses and to control the microbial load, food safety measures should be taken by following the standard protocols. In Pakistan, slaughterhouse environment, their sanitary conditions, storage, and transportation may be responsible in spreading the pathogens to food products.

Keywords: Poultry, food borne illness, antibiotic susceptibility, biochemical tests, microbial diversity, food biosafety.

INTRODUCTION: Chicken (*Gallus gallus domesticus*) occupies a distinctive place in our lives as good source of food and protein worldwide (Blake *et al.*, 2021). It is the most common and distinguished domesticated animal, distributed across continents and islands from the Arctic to the Falklands. Chickens are responsible for the development of case study in domestication. The practice began around 8000 years ago in Southeast Asia. China is recognized as the center of origin for chicken's domestication (Abdulwahid and Zhao, 2022). Depending on the nutritious value, chicken offer a more balanced and appropriate nutrient profile, predominantly amino acids, and proteins. Over time, the broiler meat business has evolved. Producing meat and meat products for human consumption is the aim of broiler farming. A significant portion of poultry production is comprised of the production of broilers, with global annual output expected to reach 105 million metric tons in 2023 and a predicted growth rate of 1.73% between 2019 and 2023 (Maharjan *et al.*, 2021). In 2018, Pakistan's share of world's meat production, poultry meat production and chicken meat production were between 1% to 1.2%. Since 1971, Pakistan's poultry sector has expanded extraordinarily in line with global trends. With a current turnover of roughly 750 billion PKR, this expansion has been sufficiently reflected to meet industry requirements. In Pakistan, poultry accounts for 40–45% of all meat consumption. Along with 1.44 billion Kg of annual meat production, the poultry industry also turns out 17.5 billion annual table eggs. The share of poultry in Pakistan GDP is around 3.1% (Memon *et al.*, 2021). The health and growth of chicken depend upon the largely diverse unexplored complex microbiological community that occupies the chicken gastrointestinal tract. The microbial community plays a critical role in maintaining intestinal homeostasis in chickens' gastrointestinal tracts, which are home to a variety of microorganisms. The intestinal flora is essential for mucosal immunity, intestinal growth, digestion, and host nutritional absorption. Chicken health like human is crucial for normal physiology. The chicken gut comprises of more than 100 trillion microorganisms that are knowns as gut microbiota These include various types of bacteria, fungi, viruses, and protozoa (Zmora *et al.*, 2019). The ileum has a more diversified bacterial community than the duodenum, in comparison to the rest of the small intestine. In the duodenum, most of the microbiota population is made up of *Lactobacilli* and *Bifidobacteria*. *Enterococci*, *Clostridiaceae* and *Lactobacilli* are typically found in the jejunum, but the ileum is dominated by *Bacteroides*, *Clostridia*, *Streptococci*, *Escherichia coli*, *Enterococci*, *Ruminococci*, and *Lactobacilli*. The ceca have a more diversified, numerous, and stable microbial community made up of *Clostridium*, *Enterococcus*, *Bacillus* and *Ruminococcus,* as compared to the ileum. The cloaca, like the caeca, has a more diverse and abundant bacterial population, which includes *Streptococci*, *Enterococci*, *Clostridia* and *E. coli* (Wickramasuriya *et al.*, 2022). Pathogenic bacteria found in chicken have a significant impact on both the poultry business and human health. Majority of the bacteria, including human pathogens like *Campylobacter*, *E. coli* and *Salmonella*, live in the avian host as passive commensals. Variations in the microbiota's make-up may have long-term effects on both the host bird and its associated products. Because dysbiosis, or imbalance in the microbiome, is linked to poor nutritional absorption, stomach irritation, and symptoms

like diarrhoea, which can spread disease when mixed with other birds. Dysbiosis can cause blooms of suspected pathogens including *C. perfringens*, which can affect both the host and human consumers, and APEC (Avian Pathogenic *E. coli*), which may be related to human ExPEC (Extraintestinal Pathogenic *E. coli*) (Clavijo and Flórez, 2018). Poultry slaughter and processing potentially transmit GIT related microbes between corpses and contaminate environment and processing surfaces, particularly during the disemboweling procedures and defeathering (Boubendir *et al.*, 2021). If the meat is not handled appropriately by the consumer, these germs could cause foodborne salmonellosis and campylobacteriosis outbreaks and reduce the quality of poultry meat (Marmion *et al.*, 2021). Contamination of poultry remains a public health issue causing serious foodborne illnesses in humans. Food producers and handlers now have a larger obligation to maintain food safety due to the changing global problems. To maintain food safety throughout the whole food chain, from production to consumption, WHO collaborates closely with the Food and Agriculture Organization (FAO), the World Organization for Animal Health (OIE), the UN Environment Programme (UNEP), and other international organisations. It offers complete guidelines and recommendations including HACCP (Hazard Analysis Critical Control Point) and Codex Alimentarius Commission (CAC). The studies undertaken by FAO and WHO on *Campylobacter* and *Salmonella* prevalence in broiler chickens have offered a risk assessment model. But still better practices are required to mitigate the risk of contamination of *Campylobacter* and *Salmonella* from processing to preparation of food (Gichure *et al.*, 2022).

OBJECTIVES: The key objectives of this study were to evaluate the bacterial diversity associated with the commercial broiler chicken in Lahore, Pakistan. The culture dependent and phylogenetic analysis of bacterial community was carried out to determine the associated health hazards. This study focused on the sanitation and hygienic conditions of the raw commercial broiler chicken meat taken from the local butcher's shops.

MATERIALS AND METHODS: In sterile plastic bags, 8 samples of commercial broiler chicken meat were collected from Shadman, Moon, Karim Block, Tollinten, Jallo and Wapda Town meat market of Lahore, Pakistan. Samples were processed immediately in the laboratory for the isolation of bacterial strains on Eosin Methylene Blue Agar (EMBA), Agar Salmonella Shigella Agar (SSA), Mannitol Salt Agar (MSA), Blood Agar (BA), Thiosulfate Citrate-Bile Salts Sucrose (TCBS Agar) and Modified Charcoal Cefoparazone Deoxycholate Agar (mCCDA). The General Viable Count (GVC) was done by performing serial dilution of the samples (10% w/v) in 0.1% peptone saline solution. All the plates were then incubated for 24 h at 37°C after spreading. Colony Forming Units per Gram (CFU/g) of the sample were calculated after incubation.

Biochemical characterization: Biochemical tests were performed for screening of bacteria (Miller and Yeung, 2022).

Catalase test: The bacterial cultures were analyzed for the detection of catalase production. A few drops of 3% hydrogen oxide were applied to a clean glass slide. A sterile toothpick was used to transfer a 24 h. old bacterial culture on the slide.

Cytochrome oxidase test: Tetramethyl-paraphenyline diamine dihydrochloride, the oxidase reagent, was formed by dissolving 1 mL of the reagent in 10 mL of distilled water. The filter paper was treated with 4 drops of oxidase reagent. With the help of sterile toothpick, 24 h old bacterial culture placed onto the filter paper at a site of oxidase reagent. After20 sec, appearance of blue color was observed for positive test results.

Methyl red test: The methyl red test is employed to identify the bacteria that can produce stable acids from the fermentation of glucose. At acidic pH of 4, methyl red changes into red indicating positive result. About 5 mL Methyl Red Voges Proskauer broth (MRVP) was prepared in test tubes. Sterile broth was inoculated with fresh bacterial cultures and incubated for 24 h at 37°C. About 50 µL of methyl red indicator was added and tubes were observed for positive result.

Voges proskauer test: Voges Proskauer test is utilized to determine the microorganisms that can produce neutral end products e.g., acetylmethylcarbinol (acetoin) from organic acids because of fermentation of the carbohydrates. The Barritt's reagent is used in this test, which is the combination of 5% α-naphthol and 40% potassium hydroxide solution. After the addition of reagent, the appearance of rose red color indicates the positive result. Each test tube was filled with 5 mL of the prepared MRVP broth, which was then autoclaved. Test tubes were inoculated with fresh bacterial cultures and incubated at 37°C for 24 h. Afterwards, 200 µL of 40% KOH and 600 µL of α-naphthol were added to each test tube and then observed for rose red color.

Citrate utilization test: Citrate Utilization test is used to identify organisms that can use citrate as a carbon source in the absence of lactose and glucose. Bromothymol blue is used as an indicator in this test and changes its color from green to blue in the presence of alkaline environment; generated by the product. Afterward, 24 h old bacterial culture was streaked onto the sterile medium surface and stabbed inoculated with the use of sterilized inoculating loop. After incubation, turbidity of the medium and change in color of the medium was recorded.

Urease test: The urease test is used to evaluate the capacity of microorganisms to break down urea in the presence of urease enzyme. It leads to the production of critical end products like toxic ammonia. About 5 mL urease broth was prepared in different test tubes and autoclaved. Fresh bacterial culture was inoculated in the broth while one tube was kept as uninoculated control. Afterward, tubes were incubated at 37°C for 24 h and color change to deep pink was observed that was taken as a positive test. Later color change was observed, and results were recorded for all strains.

Molecular characterization: The 16S rRNA gene sequencing from 19 bacterial strains was accomplished to analyze the bacterial diversity associated with the commercial broiler chicken. Genomic DNA from 63 bacterial strains was carried out by using FavorPrep™ Tissue Genomic DNA Extraction Mini Kit. The strains were then proceeded for PCR amplification of 16S rRNA gene by using 27f forward (5' AGA GTT TGA TCC TGG CTC AG 3') and 1522r reverse primer (3' ACG CC(AG) ACC TAG TGG AGG AA 5'). For PCR amplification, Thermo Scientific™ DreamTaq Green PCR Master Mix (Lot# 01025162) was used. The reaction mixture containing tubes were incubated in thermal cycler under specific conditions i.e., initial denaturation at 94°C for 5 min., denaturation at 94°C for 1 min., annealing at 55°C for 1 min. (30 cycles), extension at 72 °C for 5 min. followed by final elongation for 10 min. at 72°C. Following

purification, the PCR products were forwarded to First BASE Sequence Laboratory (Singapore) for 16S rRNA gene sequencing. Following sequencing, certain sequences were retrieved and pruned to help identify the correct bacterial species. For this purpose, CHROMAS lite Version 2.4.1.0, a Bioinformatics tool was used, and species identification was done by searching the sequence homology by using the software NCBI Basic Local Alignment Search Tool (BLAST). Finally, sequences were submitted to NCBI to obtain the GenBank accession numbers.

Phylogenetic analysis: Nineteen sequences were aligned with the help of Multiple Sequence Alignment data base (Clustal W) by using the software MEGA11 (Tamura *et al.*, 2021). The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Maximum Composite Likelihood method and in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated (complete deletion option). There was a total of 274 positions in the final dataset.

Antibiotic susceptibility testing: For antibiotic sensitivity testing Kirby Bauer or disk diffusion method was used. For this purpose, Mueller-Hinton (MH) agar medium was prepared and autoclaved. With the help of sterile cotton swab, 24 h old bacterial culture was taken, and swabbing of culture was done on MH agar plates. Afterward, the discs of antibiotics i.e., Chloramphenicol, Streptomycin, Tetracycline and Gentamycin were aseptically placed with the help of sterile syringe at well-spaced distance on the surface of the agar plates. All plates were then incubated at 37°C for 24 h. Later, the clear zones of inhibition were observed on the agar plates around the antibiotic discs. The zones of inhibition surrounding the discs were measured in millimeters (mm) using the manufacturer's provided Zone Inhibition Ruler. Then the comparison of zones with the Standardized Chart (M100-S23) for antibiotics was done that was provided by Clinical Laboratory Standard Institute.

Swarming motility: Loop full of bacterial inoculum was given in the center of the sterile L-agar plates to form a circle. Then, the plates were incubated for 24 h at 37°C. After incubation, the concentric circles or swarming motility of all the strains was observed and recorded.

RESULTS: Out of 8, seven showed the highest value of CFU/g as 7.3×10^4. The minimum was recorded in sample 2 (1.2×10). The CFU values of sample number 1, 3, 4, 5, 6 and 8 were 2.14×10^4, 2.8×10^4, 6.1×10^4, 4.3×10^4, 5.25×10^4 and 4.06×10^4, respectively. Sixty-three bacterial colonies were isolated from general and selective media (figure 1). For the 16S rRNA gene sequencing of purified strains, a total of 19 strains were sequenced from First Base Sequence Laboratories in Singapore.

16S rRNA gene sequencing: About 33 strains showed PCR positive results with 16S rRNA. The amplified PCR products were purified by Gel Extraction kit (The FavorPrep™ GEL Purification Kit) and confirmed by using the Gel electrophoresis (figure 2). The sequencing results were then analyzed using nucleotide Basic Local Alignment Search Tool (BLAST) to check the homology of the isolated strains with the existing identified sequences of bacteria for the correct identification of species of isolated strains. Maximum strains were exhibiting 99% genetic similarity with their identified species (table 1). The study and analysis indicated that the isolated strains belong to the Genera *Proteus*, *Shigella*, *Providencia*, *Escherichia*, *Citrobacter*, *Klebsiella*, *Myroides*, *Enterococcus*, *Serratia*, *Staphylococcus*, *Bacillus*, *Macrococcus* and *Kluyvera*.

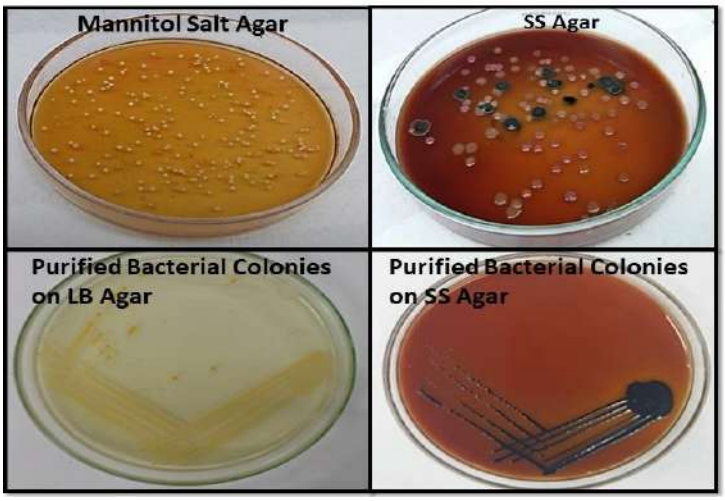

Figure 1: Growth of bacterial colonies on different medium from raw chicken meat.

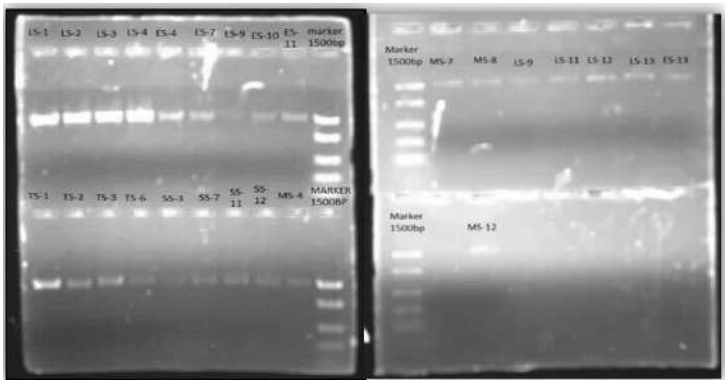

Figure 2: Agarose gel containing purified PCR products DNA bands purified for gene sequencing of 16S rRNA gene of bacterial strains.

Antibiotic susceptibility: By using that standard chart provided by the manufacture, the sensitivity of the bacterial strains was determined (table 2).

These antibiotics are broad spectrum and work against both gram-positive and gram-negative bacteria. About 7 strains showed antibiotic resistance against tetracycline, 6 strains showed resistance against Gentamycin, 3 strains showed resistance against Streptomycin. Chloramphenicol zone size was between 14 to 27 mm for all the strains. All the strains showed sensitivity against chloramphenicol. This one may be drug of choice that can be used against all microbes (figure 3).

Swarming motility detection: Ten different strains were also checked for their swarming motility on agar medium. It is movement of bacteria on a solid substrate medium by flagellates microorganisms. Four different strains of *Proteus* and one strain of *Serratia* showed the swarming motility in the center of the agar medium.

This motility was in the form of concentric circles. Strains LS-1, LS-4 and TS-6 showed the strong swarming motility with large concentric circles. Whereas LS-2 and LS-3 showed less motility and small concentric circles (figure 4).

Sr. NO	Isolates	GenBank Accessions No.	Bacterial Species	Genetic Homology (%)	Homology with GenBank Accessions No.
1	LS-1	KX417273	*Proteus mirabilis*	99%	KC456539
2	LS-2	KX417274	*P. vulgaris*	100%	KX018335
3	LS-3	KX417275	*P. mirabilis*	99%	HQ169118
4	LS-4	KX417276	*P. myxofaciens*	99%	KT362364
5	ES-4	KX417277	*Shigella sonnei*	99%	EU723822
6	ES-7	KX417278	*Providencia alcalifaciens*	99%	AY994312
7	ES-9	KX417279	*Escherichia fergusonii*	99%	HQ259938
8	ES-11	KX417281	*Klebsiella pneumonia*	99%	KU512907
9	TS-1	KX417282	*P. vermicola*	99%	KC456588
10	TS-2	KX417283	*Myroides phaeus*	99%	KM007063
11	TS-3	KX417284	*Enterococcus casseliflavus*	99%	KJ803876
12	TS-6	KX417285	*Serratia nematodiphila*	99%	KT261117
13	SS-7	KX417286	*P. hauseri*	98%	JN092599
14	SS-12	KX417287	*K. oxytoca*	99%	AJ871860
15	MS-7	KX417288	*Mammaliicoccus sciuri*	99%	KR476410
16	MS-8	KX417289	*Bacillus tequilensis*	99%	KT982227
17	LS-11	KX417290	*Macrococcus caseolyticus*	99%	KR028441
18	LS-13	KX417292	*M. caseolyticus*	100%	KT887969
19	ES-13	KX417293	*Kluyvera intermedia*	99%	KR265398

Table 1: The16S rRNA gene sequencing of bacterial strains obtained from raw chicken meat.

Sr. NO	Names of Strains	Names of antibiotics Zones of Inhibition (mm) CN	TE	C	S
1	*S. nematodiphila*	14 (I)	0 (R)	22 (S)	12 (I)
2	*P. vulgaris*	0 (R)	9 (R)	20 (S)	10(R)
3	*M. phaeus*	18 (S)	8 (R)	27 (S)	17(S)
4	*S. sonnei*	11 (R)	0 (R)	16 (I)	13 (I)
5	*Pro. alcalifaciens*	13 (I)	16 (I)	15 (I)	12 (I)
6	*B. tequilensis*	15 (S)	18 (I)	24 (S)	14 (I)
7	*K. oxytoca*	11 (R)	15 (I)	22 (S)	12 (I)
8	*E. fergusonii*	11 (R)	0 (R)	14 (I)	10(R)
9	*K. pneumonia*	11 (R)	12 R)	18 (I)	11(R)
10	*M. sciuri*	11 (R)	0 (R)	15 (I)	15(S)

Table 2: Antibiotic susceptibility patterns of bacterial isolated strains.
R = resistant, S = sensitive; I = intermediate; CN = gentamycin, TE = tetracycline, C = chloramphenicol, S = Streptomycin.

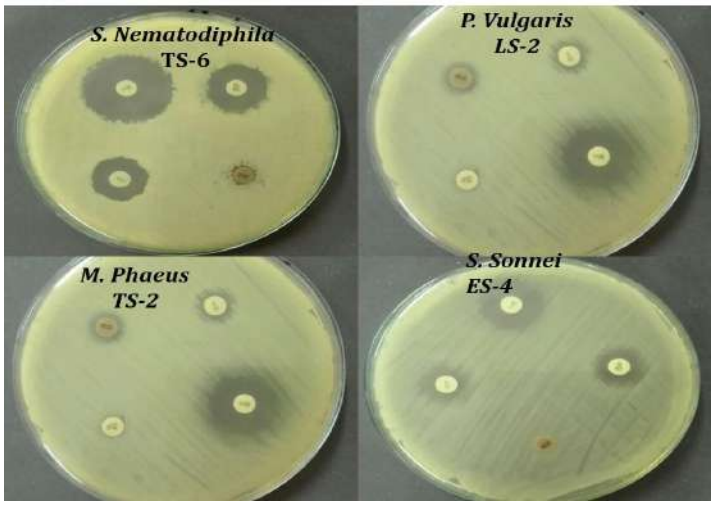

Figure 3: Antibiotic sensitivity pattern of different selected bacterial strains.

Biochemical analysis: Different biochemical test results for isolated bacterial strains are shown in table 3. For catalase test, bacterial strains ES-11, SS-12, LS-2 and LS-3 showed positive results. Only strain ES-9 showed blue color and tested positive for cytochrome oxidase test while LS-3 was positive for citrate test.

Biochemical Tests	Bacterial Strains ES-11	SS-12	LS-2	LS-3	ES-9
Catalase	Positive	Positive	Positive	Positive	Negative
Oxidase	Negative	Negative	Negative	Negative	Positive
Methyl Red	Negative	Negative	Positive	Positive	Positive
Voges Proskauer	Positive	Positive	Negative	Negative	Negative
Citrate	Negative	Negative	Negative	Positive	Negative
Urease	Positive	Positive	Negative	Negative	Negative

Table 3: Biochemical test results of different bacterial strains.

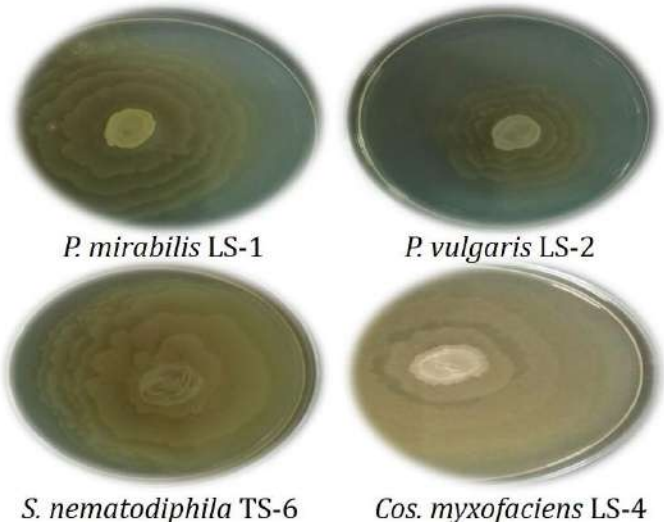

Figure 4: Swarming motility of different bacterial strains.

Phylogenetic analysis: The optimal tree constructed by MEGA11 is shown in figure 5.

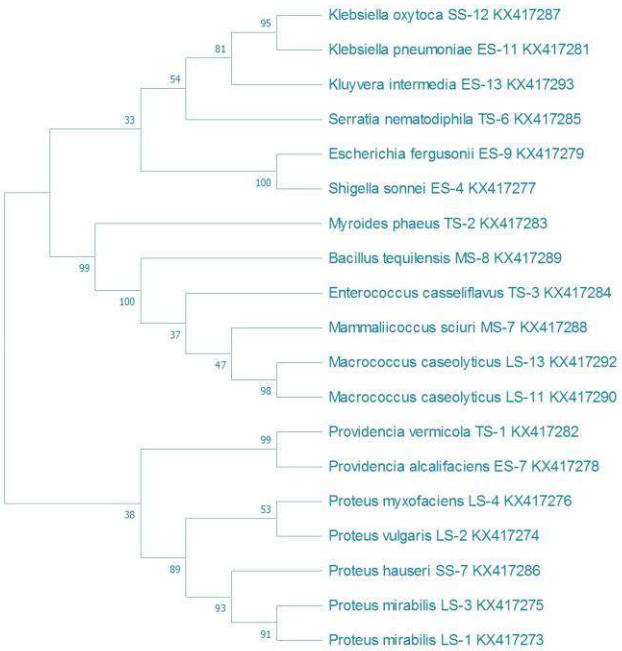

Figure 5: Phylogenetic analysis among bacterial strains.

All the gram-negative bacterial strains are clustered together, whereas gram-positive bacterial strains are clustered together in the middle of the tree. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985).

DISCUSSION: Ensuring of safe food supply is a matter of concern and a major challenge for consumers, producers, and public health organizations in both developed and developing countries (Henchion *et al.*, 2021). As the raw chicken meat is a major substrate for the growth and multiplication of different hazardous and pathogenic microorganisms causing the spoilage of food. Thus, different hygienic measures and proper sanitary methods of slaughtering and consumption of chicken meat must be ensured to avoid different diseases such as foodborne diseases that are increasing in Pakistan day by day (Ahmed *et al.*, 2021). All the samples of raw meat were showing different microbial community, majority of which were Gram negative rods. Overall, 3 samples were contaminated with *Proteus* genera. The highest microbial diversity was found in Sample 4 and 7 including the diverse genera such as *Enterococcus*, *Proteus*, *Providencia*, *Staphylococcus*, *Bacillus*, *Macrococcus*, *Kluyvera* and *Serratia* (figure 6).

In the present study, different pathogens were also isolated including *K. pneumoniae*, *E. fergusonii*, *S. sonnei*, *P. mirabilis*, *S. scuiri* and *B. tequilensis* that are all pathogenic organisms representing the contaminated food and a serious threat to human diseases and health due to unhygienic slaughtering and poor processing of chicken in street markets of Lahore, Pakistan. In a study, prospective bacteriophages (phages) infecting MDR pathogenic bacteria obtained from chickens were isolated, characterised, and their effectiveness as bio-control agents was assessed. Forty-five distinct bacterial isolates were identified in chickens, including *E. coli* (18), *Salmonellae* spp. (16), *Staphylococcus spp*. (5), *Pseudomonas spp*. (2), *P. mirbalis*, (1) *Citrobacter spp*. (1), *E. aerogenes* (1) and

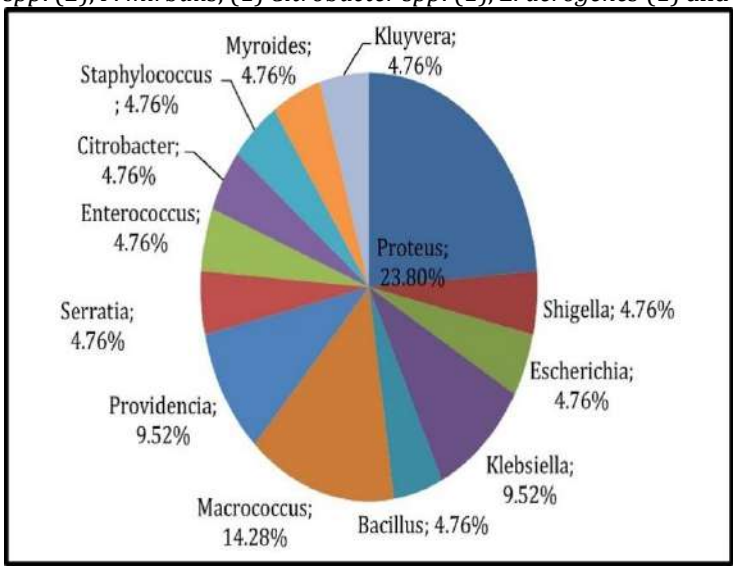

Figure 6: The percentage composition of microbial diversity associated with raw broiler chicken breast piece.

K. pneumonia (Fathy *et al.*, 2022). *Staphylococcus*, *Escherichia* and *Bacillus* spp. are associated with the severe food poisoning and foodborne diseases due to the production of toxins produced by some bacteria e.g., *B. cereus* (Bhattacharyya and Das, 2022). Rashid *et al.* (2021) confirmed microbial load variety on raw chicken meat including pathogens such as *E. faecalis*, *Bordetella spp*., *Salmonella* spp., *E. aerogenes*, *S. aureus*, *K. pneumoniae*, *Micrococcus spp*., *Citrobacter spp*., *E. coli*, *S. epidermidis*, *Proteus spp*., *K. oxytoca*, *Providencia spp.* and *Pseudomonas spp*. The prevalence of food-borne diseases is alarmingly high worldwide. It has been reported that *Campylobacter*, *Salmonella*, *E. coli* O157 and S. aureus are the most prevailing pathogens in livestock causing foodborne diseases in humans in Europe, USA and Korea (Lee and Yoon, 2021). If safety precautions are not performed, it has been noted that zoonotic meat-borne illnesses such Campylobacteriosis, Salmonellosis, *E. coli* enteritis, and food poisoning from *Staphylococcus*, *Clostridium*, etc. are the main issues faced by people who eat infected meat (Libera *et al.*, 2022). In the present study, antibiotic susceptibility pattern of selected isolated samples was studied by using the broad-spectrum antibiotics including Chloramphenicol, Tetracycline, Streptomycin and Gentamycin. Broad spectrum antibiotics work equally against both gram-positive and gram-negative organisms. Gentamycin was selected to check its effect on gram negative bacteria. The more resistance was shown by SS-12 strain that was *Myroides phaeus* and resistant against three antibiotics Streptomycin, Gentamycin and Tetracycline while the strain was susceptible to Chloramphenicol. The highest resistance was shown by the antibiotic Tetracycline that was about 45 % against all the strains (Figure 7). Hamed *et al.* (2021) reported the antimicrobial resistance in different internal organs from chicken samples. It has been observed that *E. coli*, *Salmonella* and *Staphylococcuss* spp. strains showed higher resistance to tetracycline, ampicillin, nalidixic acid, norfloxacin, streptomycin, and danofloxacin. Another study also reported high resistance of *E. coli* and *Salmonella* against doxycycline, sulfonamide, trimethoprim-sulfamethoxazole, and streptomycin (Assoumy *et al.*, 2021).

Antibiotic resistance in humans and animals is accelerating due to the irrational and improper use of antibiotics in the commercial chicken and aquaculture industries. It is important to provide farmers, feed distributors, and medicine vendors with intensive training to increase their awareness of ethical farming methods, accepted biosecurity procedures, good personal hygiene, and the appropriate use of antibiotics. The antibiotic resistance ability of microbes is a primary concern for human health in possibly pathogenic bacteria in raw chicken meat. The proper safety measures should be taken in the processing and handling of chicken to avoid diseases.

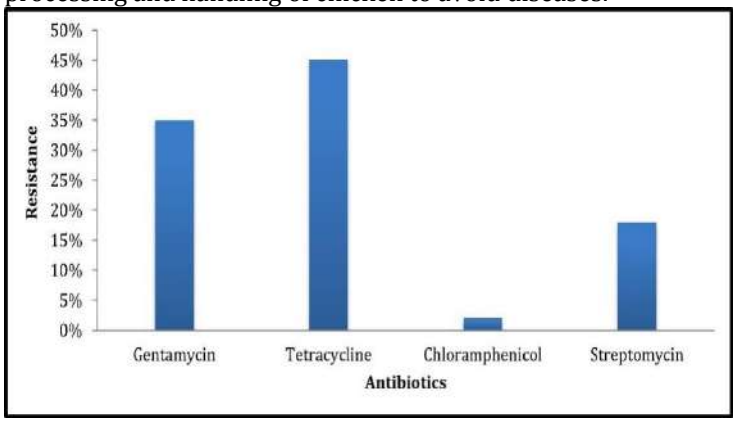

Figure 7: Percentages of antibiotic resistance prevalence in different isolated bacteria associated with raw chicken meat.

CONCLUSIONS: Finally, it was concluded that contamination of poultry remains a public health issue causing serious foodborne illnesses in humans. To overcome the foodborne illnesses and to control the microbial load, food safety measures should be followed properly by the rule of HACCP (Hazard analysis critical control point). The present study showed that chicken meat was infested by different potential human pathogens, especially, *K. pneumoniae*, *E. fergusonii*, *B. tequilensis* and *S. sonnei*. The proper handling and hygienic conditions during chicken processing may prevent the cross-contamination or transfer of potential human pathogens.

CONFLICT OF INTEREST: Authors have no conflict of interest

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