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RAPD BASED DETECTION OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM POULTRY MEAT AND WORKERS IN THE LOCAL MARKET OF PESHAWAR

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ABSTRACT

Staphylococcus aureus, a significant pathogen in humans and animals, was analyzed for genetic similarities and distances among isolates from poultry meat and workers. Fifty-one samples were collected from various poultry parts (chest, liver, gizzard, and cloaca) and workers at VRI Peshawar. Worker samples were collected via nasal swabs. Out of the 51 samples, 31 (61%) tested positive for *S. aureus*, with the highest isolation frequency from the gizzard (73%). Antibiotic susceptibility testing revealed 100% resistance to oxacillin and roxithromycin, followed by 93.5% resistance to tetracycline. Linezolid resistance was observed in 48.4% of the isolates. Genetic analysis using RAPD-PCR with primers OLP6, OLP11, and OLP13 resulted in the amplification of 130 alleles. The dendrogram based on these alleles grouped the isolates into three clusters, consisting of ten populations from chickens (C-1 to C-6) and workers (W1 to W4). Primer OLP-13 amplified the most alleles (55), while OLP-06 amplified the fewest (32). The study found varying genetic distances among and between the isolates, with maximum genetic diversity observed between certain chicken and worker isolates. The genetic relatedness of isolates from chicken meat and humans indicates potential zoonotic transmission. This research demonstrates that RAPD-PCR is an effective method for determining genetic distances and similarities among *S. aureus* isolates from poultry meat and workers, underscoring the importance of monitoring such pathogens to prevent cross-species transmission.

Key words: *S. aureus*, Antibiotics, PCR, Poultry Meat, Zoonotic,

INTRODUCTION

Staphylococcus aureus is a member of Staphylococcaceae having Gram positive cell wall, coagulase positive cocci. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates are genetically heterogeneous (URREHMAN, NAILA, & JUNAID AHMAD) . Some strains of the *Staphylococcus aureus* are more commonly present and have a tendency to spread within or between hospitals and countries. Other "sporadic" strains are present less often and do not usually spread extensively (REHMAN et al., 2023). Some clonal lineages of *S.aureus* are host specific and may be adapted to either humans or animals. Methicillin resistance in this bacterial species shows a danger to human health (J. Ahmad & Ahmad). Methicillin-resistant *Staphylococcus aureus* includes those strains that have acquired a gene giving them resistance to methicillin and basically all other beta-lactam antibiotics (Abdullah, 2022). This group of organisms has since emerged as a seriously related in human medicine (Javed et al., 2023). Methicillin-resistant *Staphylococcus aureus* was first noted as a nosocomial pathogen. The most common antibiotics are resistant to hospital associated strains and their treatment is challenging (Asif Aziz et al., 2022; Ullah et al., 2019). In food animal production antimicrobials are widely used, where they are often applied sub-therapeutically for growth and disease prevention (B. Ahmad et al., 2022). Surveys conducted by the National Antimicrobial Resistance Monitoring System show that with multidrug-resistant *Campylobacter* species, *Salmonella* species, *Enterococcus* species and *Escherichia coli* retail meat and poultry products are rapidly contaminated (Aamir Aziz, 2022; Ullah et al., 2019). *S. aureus* is among the most prevalent cause of clinical infections globally and has garnered substantial public attention due to increasing mortality associated with multidrug resistance. A new MRSA strain, ST398 has arisen that predominantly colonizes people working in food animal production. First discovered in 2003, ST398 now makes up a substantial proportion of the community-acquired MRSA cases in the Netherlands (Fatima, 2022; Shar). Methicillin-resistant *Staphylococcus aureus* was later found in broiler chickens in Belgium and in broilers but not in breeder chickens in the Netherlands**.** These samples were identified as the livestock associated strain, ST398. As per the above scenario the recent investigation was designed to evaluate the following aims and objectives (Gul, 2022). Different food can act as a good medium for *S. aureus* genes are accessory genetic elements in *S. aureus*; they such as raw meat and meat products raw milk, dairy are encoded by mobile genes. Specific PCR for products and ready-to-eat foods. Enterotoxins are detection of enterotoxin genes in food has been highly thermostable; normal cooking and pasteurization developed (Aamir Aziz, 2022). The present work was planned to cannot totally inactivate them, so they cause food investigate the prevalence of *S. aureus* in food samples poisoning. The onset of symptoms depends on the characterization of enterotoxigenic *S. aureus* isolates amount of enterotoxin ingested (PARMAR, 2001). Classic SEs antigens have been identified as SEA, SEB, SEC1, SEC2, SEC3, SED and. Recently, several other toxins were detected RPLA can identify enterotoxins using specific antibodies for each of the enterotoxins, but cross reaction between SEA and SEE have been reported Specific PCR for detection of enterotoxin genes in food has been developed (Rafique, 2023). The aim of the study to determine the prevalence of *Staphylococcus aureus* in poultry meat and workers, to evaluate the antibiotic susceptibility profiles of *Staphylococcus aureus* in poultry meat samples from Peshawar and molecular characterization of *Staphylococcus aureus* isolates from poultry meat and workers.

MATERIALS AND METHODS

Sample Collection

The current study was designed to evaluate the retail poultry meat samples from Peshawar for the presence of *Staphylococcus aureus* and its susceptibility pattern. A total of 51 samples of different parts of poultry meat (Chest, Liver, Gizzard and clocae) and sample from workers were collected from nasal from Postmortem room VRI Peshawar. The samples were collected in sterile plastic bags and transported aseptically to the laboratory of Microbiology Abasyn University Peshawar for further processing.

Culturing and Phenotypic Identification

The samples in the laboratory were surface sterilized. A cut was made on the surface of the meat. Through sterile cotton swab the samples were streaked directly on Mannitol Salt agar and incubated at 37ºC for 48 hours to get the colonies (Hayat, 2022). The golden yellow colour colonies were found which indicate the presence of *Staphylococcus aureus*. Samples from workers were be collected through nasal swabs and were streaked on the medium. The isolated organisms were subjected to biochemical test for confirmation by using previously described method by (Robina et al., 2021). The isolated organism were subjected to phenotypic and biochemical identification by using the method of. The organism was confirmed through Gram staining, coagulase and catalse tests.

Antibiotic Susceptibility Profile

For determination of antibiotic susceptibility profile of *Staphylococcus aureus*, Disc diffusion method (Kirby-Bauer method) were adapted for the commonly used antibiotics. The following anti-microbial were tested against the isolated *Staphylococcus aureus*.

Table-1: Antibiotics used against *Staphylococcus aureus*.

S.No	Antibiotics	Potency
1.	Linezolid	30µg
2.	Clarithromycin	15µg
3.	Vancomycin	05µg
4.	Novobiocin	30µg
5.	Oxicillin	01µg
6.	Fusidic acid	10µg
7.	Tetracyclin	30µg
8.	Chloramphenicol	30µg
9.	Gentamycin	10µg
10.	Amikacin	30µg
11.	Mixofloxacin	05µg

DNA Extraction

A single colony was incubated overnight in 5 ml of nutrient broth at 37ºC. Then, 1.5 ml of the culture was centrifuged at 14,000 rpm for 5 minutes and resuspended in 200 µl of lysis buffer. After adding 66 µl of 5M sodium chloride, the sample was centrifuged at 14,000 rpm for 10 minutes at 4ºC. The supernatant was mixed with chloroform, inverted, and centrifuged. The upper layer was mixed with ethanol, centrifuged, washed with 70% ethanol, dried, resuspended in 100 µl of H2O, and stored at -20ºC.

RAPD PCR

The molecular characterization was performed using RAPD-PCR following Nanvazadeh et al. (2013) under optimal conditions for *S. aureus*. Three RAPD primers (OLP-6, OLP-11, OLP-13) were used. Each 25 µl PCR reaction contained 1X PCR buffer, 2 µl template DNA, 3 mM MgCl2, 2.5 µl each dNTP, 1 µl primer, 0.2 µl Taq DNA polymerase, and water. The PCR program included an initial denaturation at 94ºC for 3 min, 30 cycles of 94ºC for 45s, 35ºC for 30s, 72ºC for 1 min, a final extension at 72ºC for 7 min, and a hold at 4ºC. PCR products were run on a 1% agarose/TBE gel, stained with ethidium bromide, and visualized under UV light.

Table 2: List of RAPD primers used in the current experiment

S. No	Sequence Of The Primer
1.	OLP6 (5′-GAGGGAAGAG-3′)
2.	OLP11 (5′-ACGATGAGCC-3′)
3.	OLP13 (5′-ACCGCCTGCT-3′)

Statistical analysis

For statistical analysis of RAPD, clearly observed bands were considered single alleles/loci, scored as present (1) or absent (0). Genetic distances were calculated using UPGMA software following Nei and Li (1979), with the formula GDxy = 1 - dxy / (dx + dy - dxy), where GDxy is the genetic distance between two genotypes, dxy is the number of common loci, dx is the total loci in genotype 1, and dy is the total loci in genotype 2. The DNA amplification profiles were analyzed using Dendro UPGMA software for genetic analysis.

RESULTS

A total of 51 samples were collected from chickens and workers to isolate *Staphylococcus aureus*. Of these, 31 (61%) were positive and 20 (39%) were negative. The highest isolation frequency was from the gizzard (73%), followed by the cloaca (56%), liver (53%), and chest (50%). Among worker samples, 70% were positive for *S. aureus* (Table 3). Various antibiotics were tested against the isolated *S. aureus* from chickens and workers.

Table 3: Frequency of *S. aureus* from Chickens and Workers

S.No	Source of Swab	Total	Positive (%age)	Negative(%age)
1	Gizzard	11	8 (73)	3(27)
2	Liver	15	8(53)	7(47)
3	Chest	6	3(50)	3(50)
4	Cloaca	9	5(56)	4(44)
5	Workers	10	7(70)	3(30)
6	Total	51	31(61)	20(39)

Gizzard

In the gizzard, *Staphylococcus aureus* showed 100% resistance to oxacillin, tetracycline, and roxithromycin. Resistance to chloramphenicol, fusidic acid, clarithromycin, and novobiocin was 87%, to amikacin 75%, to gentamycin 62%, and to linezolid 38%. Cephalothin had the lowest resistance at 13%, with amikacin and gentamycin showing 13% intermediate resistance.

Liver

In Liver Oxacillin, Tetracyclin, Roxithromycin,Novobiocin were noted for higher resistance 100%which is followed by Moxyfloxacin, Clarithromycin 87% Fusidic acid 75% , Clorompheni̇col, 62%, Gentamycin 50%, Amikacin and Lenzolid 38%. Cephalothin were noted Low resistance 13% .Amikacin 37%, Clorompheni̇col and, Gentamycin were noted intermediate 13% .

Chest

The *S. aureus* isolated from chicken meat (chest) were subjected to different antibiotics for antibiotic resistance profile. Different level of resistance was noted for different antibiotics used in the study (Ta. In chest Oxicilline, Clarithromycin, novobiocin, Roxithromycin showed high resistance 100% which is followed by Moxyfloxacin, Lenzolid and Tetracyclin 67%. Whereas Clorompheni̇col, Amikacin, Fusidic acid, Gentamycin 33% were noted low resistance to the samples. Amikacin, Tetracyclin 33% and Clorompheni̇col, 32% were intermediate.

Cloaca

The data regarding the antibiogram analysis of cloacal samples is represented in Table-7. In cloacal isolates Oxicilline and Roxithromycin were noted for 100% resistance. Table-6 also shows that the antibiotics Amikacin, Fusidic acid, Gentamycin, Tetracyclin Clarithromycin, Novobiocin were 80%

resistant to the isolates from cloaca. Similarly antibiotics Cephalothin (60%) and Lenzolid (40%), Moxyfloxacin (20%) were observed for low resistance as compared to Oxicilline and Roxithromycin

Workers

The antibiogram analysis of the *S. aureus* isolates from workers. Most of the antibiotics were found resistant to the isolates. The data also indicates that Amikacin, Fusidic acid, Moxyfloxacin, Tetracyclin, Clarithromycin Oxicilline, Roxithromycin were 100% resistance followed by Clorompheniсol, Novobiocin 86% and Lenzolid 71%. Gentamycin were Low resistance 43%. Cephalothin 29%, Gentamycin and Novobiocin 14% were noted Intermediate. MRSA was prevalent in gizzard, liver, chest, and cloaca with 100% resistance to Oxicillin. Clarithromycin showed varied resistance: 71.4% in feeders, 60.0% in feet, and 33.3% in drinkers. Overall, Oxicillin and Roxithromycin exhibited highest resistance (100%), followed by chloramphenicol, amikacin, fusidic acid, and gentamycin (33%); moxifloxacin had 20% resistance.

Table-4: Over All antibiogram analysis of different antibiotics against *Staphylococcus aureus* isolates.

OverAll Antibiotic sensitivity				
S.No	**Antibiotics**	**R(%)**	**S(%)**	**I(%)**
1	Chloramphenicol (C)	24(77)	2 (6.5)	5(16.1)
2	Amikacin (AK)	21(67.7)	6(19.4)	4(12.9)
3	Fusidic acid (FD)	25(80.6)	0	4(12.9)
4	Moxyfloxacin (MXF)	22(70.1)	1(3.24)	0
5	Lenzolid (LZD)	15(48.4)	0	16(51.6)
6	Oxacillin (OX)	31(100)	0	0
7	Cephalothin (KF)	5(16.1)	2(6.5)	24(7.7)
8	Gentamycin (CN)	16(51.6)	4(12.9)	11(35.5)
9	Tetracyclin (TE)	29(93.5)	1(3.24)	1(3.24)
10	Clarithromycin (CLR)	28(90.3)	0	3(9.68)
11	Novobiocin (NV)	28(90.3)	1(3.24)	2(6.45)
12	Roxithromycin (RL)	31(100)	0	0

Molecular Characterization

A total of 32 scorable alleles were amplified by primer OLP-06 in *S. aureus* isolates from chickens and workers. Population C-1 showed the maximum number of alleles, while population W-2 exhibited only one allele. Table 10 shows varying genetic distances among the isolates, with the highest diversity (66%) observed between chicken and worker isolates. Chicken isolate C-1 had a genetic distance of 16% from worker isolates W-2 and W-3.

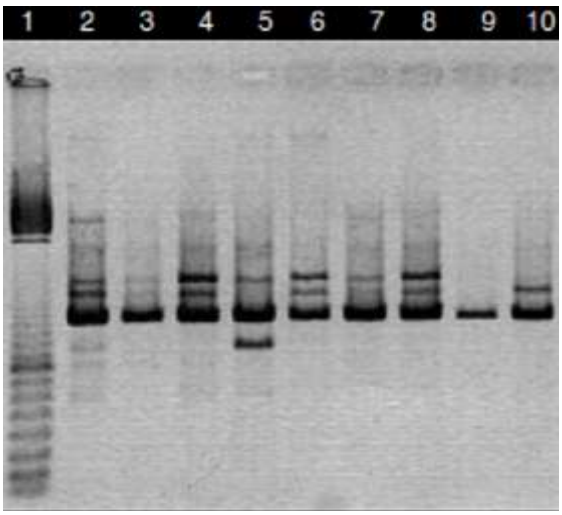

Fig1. DNA fingerprinting result for primer OLP -6.

Allele scoring data for *S. aureus* isolates amplified by RAPD primer OLP-11, totaling 43 alleles. Primer OLP-6 amplified 7 alleles in chicken isolates C-1 and C-2, and a minimum of 2 alleles in C-6, W-1, W-2, and W-4. Genetic distances varied among isolates; maximum distance (100%) was observed between C-3, W-1; C-6, W-2; W-1, W-2; and W-2, W-4. Notably, no genetic distance was calculated between C-1, C-2 and C-6, W-4 using primer OLP-11.

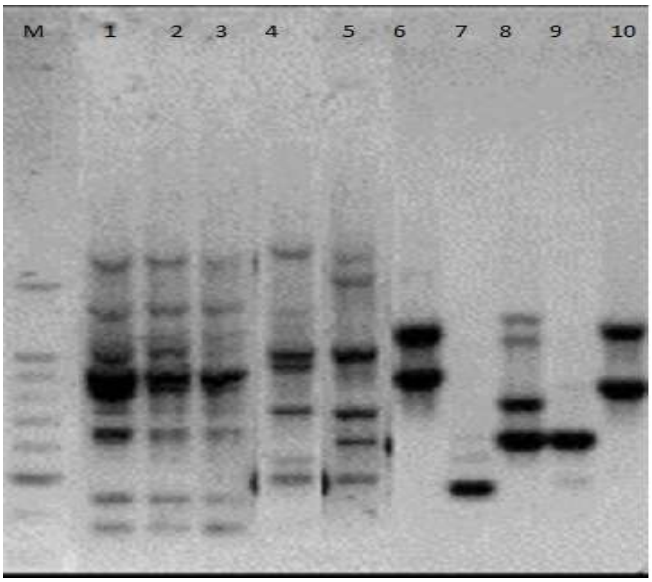

Fig.1. DNA fingerprinting result for primer OLP -11.

The primer OLP-13 amplified 55 alleles in S. aureus isolates from chickens and workers. Maximum alleles (7) were found in C2, C3, and W2, while only one allele was amplified in C-6. Allele counts, with W-1 having 5 alleles and W-4 having 4 alleles. Genetic distances tabulated reveal varying levels of relatedness: no distance (0%) was noted for C-2; C-3, C-2; W-2, C-3; W-2, and C-4; C-5, while the maximum distance (85.7%) was observed between C-2; C-6 and C-3; C-6 isolates.

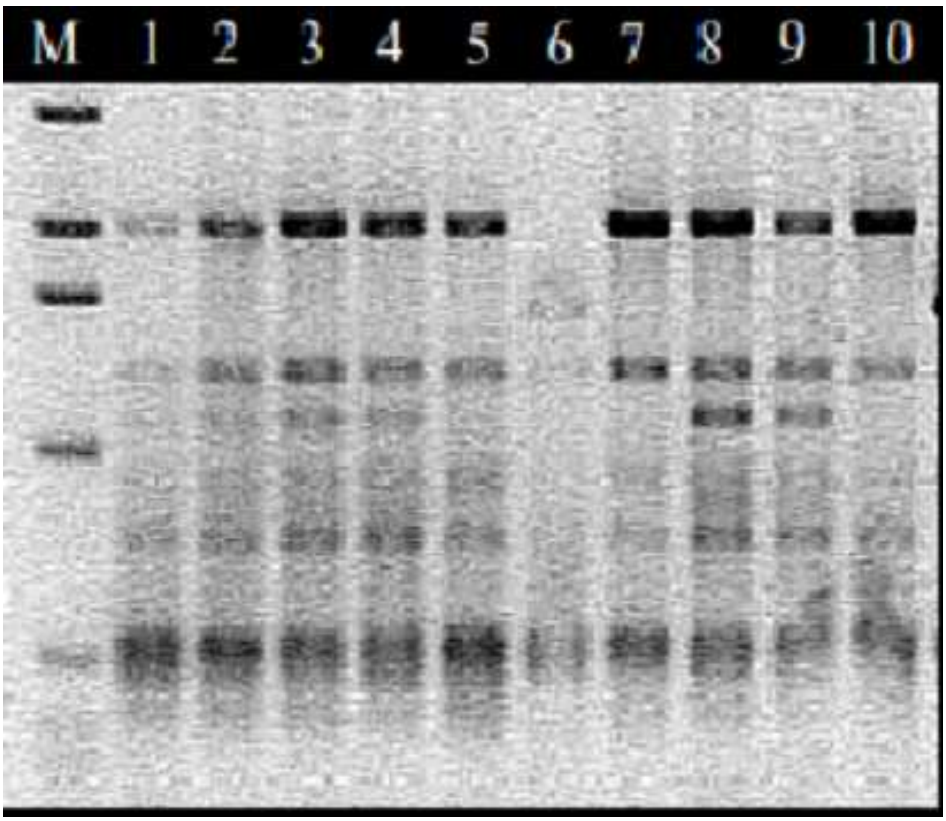

Fig.3. DNA fingerprinting result for primer OLP -13.

It presents the overall genetic distances among isolated *S. aureus* from chicken meat and workers, showing varying levels of genetic diversity. The highest distances were observed between C5 and C6 (0.77), C6 and W2 (0.7), and C3 and W1 (0.61). Similarly, a distance of 0.61 was noted between C5 and W4. The least distance (0.20) was found between C1 and C3, both from chickens. The table also highlights diverse genetic distances between isolates from workers and chickens.

Cluster analysis and over all statistics of three RAPD primers

In Figure-4 shows the genetic dissimilarity matrix of S. aureus genotypes based on three RAPD primers, clustering ten populations from chickens (C-1 to C-6) and workers (W1 to W4) into three main clusters. Cluster 1 includes four populations (C1, C2, C3, C4) from different parts of chickens, with C1 closely related to C2 and C3. Population C-4 clusters separately, slightly different from C-1, C-2, and C-3. Cluster 2 further divides into three subclusters, including populations C-5, C-6, W-1, W-2, and W-3. C-5 and W-1 are closely related in subcluster 1, while W-3 is distinct in subcluster 2. Subcluster 3 contains closely related populations C-6 and W-4, distinct from other chicken and worker isolates. Worker isolate W-2 forms a separate cluster on its own in the dendrogram.

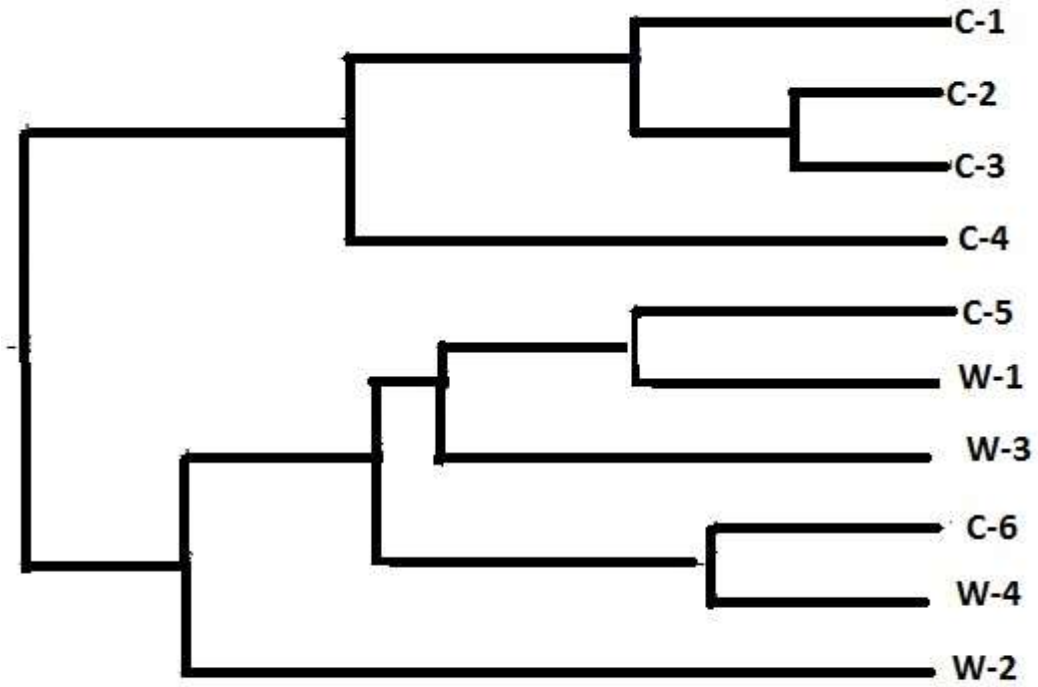

Fig.4. RAPD-PCR analysis of *Staphylococcus aureus* strains isolated from chicken and workers.

DISCUSSION

Styphylococcus aureus is the leading cause of nosocomial infections and is responsible for a wide range of human diseases, including endocarditis, food poisoning, toxic shock syndrome, septicemia, skin infections, soft tissue infections and bone infections, as well as bovine and ovine mastitis (PARMAR, 2001). Serious *Styphylococcus aureus* infections caused by strains that are methicillin resistant (MRSA)or susceptible and which may or may not express the pathogenic Panton- Valentine leucocidin (PVL) toxin, also MRSA are cross resistant to all currently licenced- β lactam antibiotics. Throughout the study 51 samples were collected aseptically from chickens and workers for isolation of *Staphylococcus aureus*. The study revealed that 31 (61%) cases were recorded positive. Our colonization rate for *S.aureus* were agreed with some other studies like 52.04%, 50% ,while findings of this study were higher as compare with Akbar and (Hassan, 2017) 18.18% and (LECTURE, 2012) who reported that 40% of the poultry meat samples contain *S. aureus.* Enhanced contamination of rate of *S. aureus* is a principal reason for the inadequate microbiological quality of chicken meat available in general market. Antibiogram was also constructed against different antibiotics for the isolated *Staphylococcus aureus*. According to Table-9 linezolid was susceptible to all of the isolated *S. aureus.* Almost similar result was reported by (Rashid, 2023). A most prominent finding in the current experiment is the detection of of *S. aureus* strains resistant to most of the tested antibiotics. These result may be due to the presence of other resistant or genetic markers or due to production of β-lactamases; or maybe the isolates were MRSA: as methicillin-resistant *S. aureus* has been observed to be resistance to almost all antibiotics, PBP2 a proteins which observed low binding affinity for β-lactams (Ali, 2012)**.** This finding may likely portend further difficulty in treatment of *S. aureus* infections in both humans and animals, as this pathogen is becoming a major danger in both human and veterinary medicine. In a study in the US, (Jessie, 2019) studied *S. aureus* from the joints and some other organs like liver, gizzard etc of diseased chickens and find out that the isolated pathogens were susceptible to Ampicillin, Penicillin and ciprofloxacin. In our study different level of sensitivity and resistance was noted for different antibiotics used. Among the tested antibiotics oxicillin shows

that 100% isolates were resistant to this antibiotic. Although MRSA strain has always shown multi resistance (Karp et al., 2017). We found that most of the isolates of the current experiment also shows resistance to multipal antibiotics. Our isolates were highly resistant to oxacillin (100%), Roxithromycin (100%) followed by tetracycline (93.5%), Clarithromycin and Novobiocin (90.3%). The results of our study are somehow different form that reported by (Khan et al., 2022) reported resistances to tetracycline (67%) and erythromycin (30%) from retail pork and beef in Louisiana. There is existence of host specificity in different bacterial pathogens Martinez et al., (2000). There are different molecular techniques have been used to differentiate *S. aureus* form animals and humans to non-host and host biotypes (Waheed, Suleman, Mirza, & Lössl, 2022). Molecular markers are used to find out genetic diversity in different types of organisms. The isolated *S. aureus* were subjected to molecular characterization through three RAPD primers. When RAPD-PCR profiles were analyzed, most of the samples showed resemblance with each other as reflected from dendogram. Three clusters were formed by the three RAPD primer consist of 6 samples from chickens and 4 from workers. Cluster-1 totally belong to the *S. aureus* isolates form chickens whereas in cluster-2 and Cluster-3 the isolates were distributed as evident from Figure-4 Different level of genetic distances were observed among and between the *S. aureus*. A total of 130 Alleles were amplified by the three RAPD primers used in the study. This numbers of Alleles were higher in the study of (SAEED & MOHAMMAD, 2021). They reported a total of 69 Alleles amplified by the RAPD primers used to differentiate between bovine and human types of *S. aureus*. Maximum no of Alleles (55) were amplified by RAPD Primer OLP-13, whereas minimum (32) Alleles were amplified by the Primer OLP-06. Previous studies reported different numbers of Alleles.

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