Antimicrobial drugs are regularly used to treat the animals against various diseases for the survival of animals. Unfortunately, residues of these antibiotics persist in meat due to the lack of knowledge about proper dose, quantity, side effects and withdrawal time from the body system of the animals. The consumption of meat containing antibiotic residues has severe negative consequences on human health and quality of life (Beyene, 2016). Antibiotics are used at vast scale in all types of meat industry, livestock, dairy and poultry due to their easy availability at cheaper rates and effectiveness (Novais et al., 2010).

The antibiotics are administered to animals through various methods (intramuscularly, orally and dietary via food) and thus retain in different body sites of animals accordingly (Berends et al., 2001). Presence of antibiotic residues in the meat of animals sold for human consumption has become a matter of public concern due to their potential hazardous effect on human health when antibiotic residues gain entry into the human food chain. The illegal and frequent use of veterinary drugs causes buildup of antibiotic residues in meat and other animal driven products that pose serious health hazards to consumers (Prajwal et al., 2017). Recently, the safety of antibiotic residues in the environment has become a matter of increasing public scrutiny and legal requirements (Dahshan et al., 2015).

Microbial inhibition tests are used to detect the antibiotic residues in meat and other products of animals as a screening method, i.e. STAR (Screening Test for Antibiotic Residues) (McGlinchey et al., 2008; Cristofani et al., 2009). The other screening methods are carried out by employing various approaches, i.e., chromatic and immunological (Wang et al., 2017; Sophila et al., 2018). Microbial screening methods are considered suitable for the determination of antimicrobial drug residues because they are cost effective and less expensive than chromatographic and immunochemical methods. The objective of the present study was to detect the antibiotic residues in the meat samples collected from different slaughterhouses of Lahore.

MATERIALS AND METHODS

The beef samples for the present study were collected from commercially important slaughterhouses of Lahore (M. Younaf & Sons, PAMCO, Baker Mandi slaughterhouse and Zenith slaughterhouse) and beef
samples were collected from November, 2016 to April, 2017. A total of 140 samples were collected from selected slaughterhouses. The 35 meat samples were collected from each sampling site. Samples were brought to the Department of Zoology, Lahore College for Women University, for further processing.

Procedure for detection of antibiotic residues in beef samples: Microbial inhibition test, a qualitative field disc assay was employed by using Nutrient agar by following the method as described by Hakem et al. (2013). A small amount of bacterial strain, *Bacillus subtilis* (Fig. 1) was inserted into the nutrient broth. After 24 hours, the material was spread on the nutrient agar plates and the small disc shaped samples were evaluated for the detection of drug residues by estimating the diameter of zone of inhibition (Malki et al., 2013).

The presence or absence of a transparent zone (Fig. 2) around beef samples was used as an indicator of antibiotic residues in examined samples. Beef samples were considered positive if zone of inhibition around the samples was detected after incubation of 24 hours, while without transparent zone, samples were considered negative for presence of antibiotic drug residues.

RESULTS

During the current study, 70% of beef samples (n=98) sold at different slaughterhouses for human consumption showed the presence of antibiotic residues. The results showed considerable contamination of beef with antibiotics and after the administration of antibiotics, the animals were slaughtered earlier before their withdrawal time for the particular drug.

The highest number of cases contaminated with antibiotic residues was observed in the beef samples collected from the Bakarmandi slaughterhouses, whereas the lowest was recorded in the samples of PMACO slaughterhouses with a percentage of 28.57%. Beef samples (n=35) collected from Baker Mandi slaughterhouse demonstrated 26 samples with positive results that was 74.28% whereas 9 samples showed negative results. Samples of beef muscle tissue collected from PAMCO revealed 23 samples positive with presence of antibiotic residues, whereas 10 beef muscle tissues collected from the Zenith slaughterhouse after examination by microbial inhibition test exhibited absence of transparent zones indicative of negative results with no antibiotic residues. Out of 70%, the percentage contamination of antibiotic residues in the positive beef samples of different slaughterhouses of Lahore i.e. Baker Mandi, PAMCO, Zenith, M. Yousaf & Sons were recorded as 74.28, 28.57, 37.14 and 68.57%, respectively (Fig. 3).

The present study revealed that 30% of the samples (muscle tissue) appeared negative with no antibiotic residues after examination by field disc method and exhibited no transparent zones around beef sample discs. The lowest number of beef samples containing antibiotic residues was recorded from the Bakamandi (25.72%). The percentage of negative samples without any antibiotic residues ranged from 25.72-71.43% for all the four slaughterhouses of Lahore (Fig. 4).
Drugs residues in meat and other commodities are a growing community health concern due to negligence of proper handling, especially in countries where rules and regulations implemented by official quality assurance authorities for use of antibiotics are bypassed. Consumers are conscious about contamination of meat items by drugs, pesticides and antibiotics due to their potential health hazards carcinogenicity, allergic reactions and increased bacterial resistance in humans (Ferrini et al., 2006; Mangsi et al., 2014).

During present study, detection of residues of antibiotics in the collected meat samples of four slaughterhouses (Bakermandi, Pamco, Zenith and M. Yousaf & Sons) of Lahore was performed by Microbial inhibition test. 98 samples out of 150 showed the positive results, i.e., the presence of antibiotic drug residues by showing the zone of inhibition around the samples after incubation of 24 hours. 42 samples did not show any change in them, so they were considered negative for the presence of antibiotic drug residues.

The residual contamination appeared high at the Bakarmandi slaughterhouse with 74.28% positive cases, whereas, it was lowest at PAMCO (28.57%) followed by Zenith (37.14%). The positive cases showing the presence of antibiotic residues at Bakarmandi (74.28%) and M. Yousaf & Sons (68.57%) might indicate that the animals were not held long enough before slaughter or the source of animals at these facilities is different. The lack of lairage facility might be the crucial factor responsible for a higher percentage of positive cases. Hakem et al. (2013) also reported 124 cases out of 145 meat samples with high percentage (85.51%) of positive cases having antibiotic residues due to non-compliance of the withdrawal period after antibiotic administration. Ramata et al. (2013) analyzed 150 raw meat samples and 56% were tested positive for antibiotic residues.

The present study demonstrated a high contamination of beef samples with antibiotic residues with 70% positive cases. The results of this study are in agreement with Hamdi et al. (2008) who reported 50% positive cases of meat samples containing antibiotic residues. The percentage of positive cases with antibiotic residues showed significant (P<0.05) variations in different studies carried out in various countries as Sanz et al. (2015) tested 53% antibiotic residues positive cases in Spain; Randrianomen et al. (2006) reported 36.72% positive cases. However, a low percentage of 9.8% has been reported by Alamedji et al. (2004) in Senegal and 9.56% by Saitanet al. (1993) in Thailand. The similar results were reported by Al Ghamdi et al. (2000) concerning the quantification of residues of tetracyclines in poultry products in the Eastern Province of Saudi Arabia.

Emergence of more virulent and new disease causing strains of bacteria and human health issues due to consumption of meat contaminated by antibiotic-resistant strains necessitate surveillance and detection of antibiotic residues in meat and meat products to save humans from worst consequences (Wulf and Voss, 2008; Price et al., 2012; Ewers et al., 2012; Liu et al., 2016). During current investigation, 70% of beef samples collected from different slaughterhouses of Lahore exhibited significant antibiotic residues in meat put on the market for human consumption. Babapour et al. (2012) reported 22.8% antibiotic residues after screening 500 beef and mutton samples. However, high level of antibiotic residues and elevated contamination noted in beef samples during present study might be attributed to frequent, prolonged and uncontrolled use of antibiotics by local farmers and livestock farmers at livestock farms without proper guidance and consultation of veterinarians against pathogens to treat the infections.

The high levels of antibiotic residues in beef are also indicative of the early slaughtering of animals without following the recommended withdrawal periods after administration of antibiotics to animals and lack of lairage. Ezenduka et al. (2014) also detected antibiotic residues in 42 cases out of 70 with prevalence percentage of 60% in poultry meat by microbial inhibition test. The results are in close agreement with Agmas and Adugna, 2018. Detection of antibiotic residuals might be noted due to before time slaughtering of animals without following withdrawal period recommended for human safety.

Conclusions: Beef samples of all the under-study slaughterhouses of Lahore i.e., Bakermandi, PAMCO, Zenith and M. Yousaf & Sons demonstrated high level of antibiotic residues. Considerable contamination of meat sold at these slaughterhouses might be due to inappropriate management practices, lack of lairage facility and withdrawal periods of the antibiotics after treatment were not followed by the local livestock farmers.

Authors contribution: FM designed the research. AZ, SS and AK conducted the research. FM, GJ and TK analyzed the data and wrote the article. Manuscript was read carefully by all the authors.

REFERENCES

Agmas B and Adugna M. 2018. Antimicrobial residue occurrence and its public health risk of beef meat in Debre Tabor and Bahir Dar, Northwest Ethiopia, Veter World 11:902-8.

Babapour A, Azami L and Faratsmehre J 2012. Overview of antibiotic residues in beef and mutton in Ardebil, North West of Iran. World Appl Sci J 19:17-22.

Bada Alamedji R, Cardinal E, Biagui C, et al., 2004. Recherche de résidus de substances à activitéantibactériennedans la chair de pouletconso nméenndéans la région du Dakar (Sénégal) ». Bull. Acad. Vét France, 2004 - Tome 157 - N°2.

Berends BR, Bogard V, Van Knappen AE, et al., 2001. Human health hazards associated with the administration of antimicrobials to slaughter animals. Part I. An assessment of the risks of residues of tetracyclines in pork. Vet Quar 23:2-10.

Beyene T. 2016. Veterinary drug residues in food-animal products: its risk factors and potential effects on public health. J Vetter Sci Technol 7:2-5.

Cristofani E, Antonini C, Tovo G, et al., 2009. A confirmatory method for the determination of tetracyclines in muscle using high-performance liquid chromatography with diode-array detection. Anal Chim Acta 637:1-2.

Prajwal S, Vasudevan VN, Satdu T, et al., 2017. Antibiotic residues in food animals: Causes and health effects. Pharma Innov J 6:1-4.

Dahshan H, Abd-Elali A, Megahed MM, et al., 2015. Veterinary antibiotic resistance, residues, and ecological risks in environmental samples obtained from poultry farms, Egypt. Environ. Monit Assess 187:2.

Ewers C, Bedde A, Semmler T, et al., 2012. Extended-spectrum beta-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: a global perspective. Clin Microbiol Infect 18:646-55.
Ezenduka EV, Ike OS and Anaelom NJ. 2014. Rapid detection of antimicrobial residues in poultry: A consequence of non-prudent use of antimicrobials. Health 6:149-52.

Ferrini AM, Mannoni V and Aureli P. 2006. Combined Plate Microbial Assay (CPMA): a 6-plate-method for simultaneous first and second level screening of antibacterial residues in meat. Food Addit Contam 23:16-24.

Hakem A, Titouche Y, Houali K, et al. 2013. Screening of antibiotics residues in poultry meat by microbiological methods. Bulletin UASVM, Vet Med 70:77-82.

Hamdi TM. 2008. Recherche des substances antimicrobiennnes dans les viandes. Communication au 6èmes journées des Sciences Vétérinaires. ENV 19-20 Avril, 2008.

Hakem A, Titouche Y, Houali K, et al. 2013. Screening of antibiotics residues in poultry meat by microbiological methods. Bulletin UASVM, Vet Med 70:77-82.

Liu YY, Wang Y, Walsh TR, et al. 2016. Emergence of plasmid-mediated colistin resistance mechanism mcr-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 16:161-8.

Mangsi AS, Khaskheli M, Soomro AH, et al. 2014. Detection of antimicrobial drug residues in milk marketed at different areas of Sindh province. IOSR J Agri Vet Sci 4:65-9.

McGlinchey TA, Rafter PA, Regan F, et al. 2008. A review of analytical methods for the determination of aminoglycoside and macrolide residues in food matrices. Anal Chim Acta 624:1-15.

Novais A, Comas I, Baquero F, et al. 2010. Evolutionary trajectories of beta-lactamase CTX-M-1 cluster enzymes: predicting antibiotic resistance. PLoS Pathog 6:726-35.

Price LB, Stegger M, Hasman H, et al. 2012. Staphylococcus aureus CC398: host adaptation and emergence of methicillin resistance in livestock. MBio 3:e00305-11.

Ramatia T, Ngoma L, Adetunji M, et al. 2017. Evaluation of antibiotic residues in raw meat using different analytical methods. Antibiotics 6:34-52.

Randrianomen J. 2006. Investigation sur la présence de résidus antibiotiques dans les denrées alimentaires d’origine aviaire commercialisées à Antananarivo (Madagascar): cas du muscle et de foie. Thèse de Docteur d’Etat en médecine vétérinaire. Faculté de médecine de pharmacie et d’odontostomatologie, Dakar, Sénégal.

Saitanu K, Amonsilp A, Kondo F, et al. 1993. Detection and identification of antibiotic residues in swine tissues. Proc 11th Inter Symp WAVFH, 24-29 Oct.

Sanz D, Razquin P, Condon S, et al. 2015. Incidence of antimicrobial residues in meat using a broad spectrum screening strategy. Euro J Nutr Food Safe 5:156-65.

Sophila JR, Raj GD, Kumanan K, et al. 2018. Microbial inhibition assay for detection of antibiotic residues in chicken meat using vegetative form of Geobacillus stearothermophilus. Pharma Innov J 7:753-7.

Wulf M and Voss A. 2008. MRSA in livestock animals- an epidemic waiting to happen? Clin Microbiol Infect 14:519-21.