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PREVALENCE AND ANTIBIOGRAM OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM THE FECAL MATERIAL OF POULTRY IN PAKISTAN.

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Abstract
Staphylococcus aureus is an opportunistic pathogen of humans and animals, and it is among the top 5 causes of food-borne illnesses globally. Very limited work has been reported about the prevalence of *S. aureus* and its antibiogram in the poultry industry of Pakistan. Therefore, the objectives of this study were to determine the prevalence of *S. aureus* in the healthy chicken sold in major cities of Pakistan and to determine the antimicrobial resistance pattern of *S. aureus*. The sample size was 372 and they were collected from major cities of Pakistan during 2020-2021. The overall prevalence of *S. aureus* was 41%. Antibiotic susceptibility testing of 45 isolates of *S. aureus* was carried out. For antibiotic susceptibility testing, the Kirby-Bauer disc diffusion method was used and for quality control, ATCC (25922, 25923) strains were turned into account, and results were analysed according to CLSI-2017 in WHONET software. Selected *S. aureus* isolates were found to be highly resistant against ceftazidime (98%), nalidixic acid (91%), erythromycin (87%), clarithromycin (84%), and tetracycline (82%) respectively. All samples showed high sensitivity against ampicillin-sulbactam, amoxicillin-clavulanate, amikacin, linezolid, minocycline, and rifampin with a value ranging from 100-96%. Most of the isolates were not resistant to methicillin, only 4% were MRSA and 9% showed intermediate value. Continual surveillance of antibiotic susceptibilities is eminent for the isolates obtained from healthy as well as diseased birds of poultry in establishing the baseline resistance patterns along with devising suitable AMR containment measures for the future.

Introduction
Staphylococcus aureus is a commensal of humans and animals. It dwells in the nostrils of approximately 20% of the human population and in the mucosal membrane of domestic and commercially raised poultry birds [1]. It is one of the most prevalent opportunistic pathogens and causes invasive and indubitably life-threatening infections in animals like mastitis, urinary tract infections and arthritis [2]. In humans, it is the 3rd most common cause of food poisoning mainly due to atrocious hygienic conditions [3,4]. Infections caused by *S. aureus* have been reported in different type of meat like raw chicken, turkey, veal, beef, pork, lamb, and rabbit all over the world

[5,6]. *S. aureus* was found to be most prevalent in turkey followed by chicken, veal, pork, and beef 35.3%, 16.0%, 15.2%, 10.7% and 10.6% respectively, in Netherland [7]. While in Germany, its prevalence was reported in 37.2% of samples of poultry food products [8]. In the USA, it was detected in 42.1% of poultry samples [9]. While in other countries, its prevalence was subsidiary e.g., in Canada, Italy, and Spain, its prevalence was 6.4%, 3.8%, and 1.6% respectively [10]. From Asia, only a few reports are available, and mainly these reports are of livestock-associated *S. aureus* infections [11]. In Pakistan, *S. aureus* was found in 38 samples out of 209 with an 18.18% in poultry [12].

S. aureus is the prominent cause of many infections in the chicken like synovitis, osteomyelitis, and cellulitis [13, 14]. In humans, it is the main reason for a wide range of severe infections with high morbidity and mortality rates (64%) [15]. It is renowned for its ability to develop resistance to antibiotics. Center for disease control (CDC) stated that 2.8 million people acquire antibiotic-resistant infections, and this results in the death of more than 3,500 individuals [16]. In addition to this, antimicrobial resistance is causing financial loss to the poultry industry because poultry birds have developed resistance to antibiotics. Food has a substantially significant role in the transmission of antibiotic resistance with reference to antibiotic residues and the relocation of antibiotic-resistant genes from food microflora to pathogenic bacteria [17]. Ample use of antibiotics in animal farming is the prime cause of the prevalence of drug resistance among foodborne pathogens mainly in *Salmonella*, *Staphylococcus spp.* and *E. coli* [18, 19, 20]. It is a widely accepted notion that antibiotics provided in the diet generate selective pressure on the microbial flora that in return facilitates the persistence transfer of antimicrobial resistance determinants among bacterial species which leads to the emergence of multi-drug resistant bacteria [21, 22, 23]. This results into a very few treatment options for the infections caused by antibiotic resistant microorganisms particularly *S. aureus* and methicillin resistant *Staphylococcus aureus*.

Many researchers have isolated (MRSA) from poultry because of the excessive use of antibiotics in the poultry sector [24]. Pakistan is a developing country; poultry rearing is done under stringent conditions to ensure food production at a lower cost and consequently suffers substantially from antibiotic resistance which should be the major concern for Pakistan along with entire human/animal population [25]. Despite the fundamental consequences of AMR in poultry, our knowledge about antibiotic resistance in *S. aureus* is rudimentary, ergo quality research is needed. The present study is aimed to determine the prevalence and antimicrobial susceptibility of *S. aureus* isolated from the fecal material of poultry collected from all over Pakistan because fecal material obtained from the caecum of healthy chicken is the best choice for the isolation of *S. aureus* as chicken gut microbiota serves as a reservoir of antibiotic-resistant determinants [26, 27]. It is important to monitor the evolution of AMR in poultry bacterial pathogens to devise suitable AMR containment measures for the future, so that AMR does not transpire into public health problem.

Materials and Methods

The study was carried out at National Reference Laboratory for Poultry Diseases (NRLPD), Animal Sciences Institute (ASI), National Agricultural Research Centre (NARC), Islamabad. Samples were collected and received at the laboratory under the AMR surveillance program in poultry.

Sample collection and processing

Fecal samples of healthy broilers from live bird markets were collected from different areas of Pakistan from September 2020 to March 2021. Fecal samples of these birds were transported to the laboratory within 24 hours of the collection and processed immediately. For processing, enrichment of each fecal sample was carried out in buffer peptone water (BPW).

Isolation, purification, and biochemical characterization of *Staphylococcus aureus*

Mannitol Salt Agar (MSA) was used as a selective and differential medium for the identification of *S. aureus*. For its purification purpose, distinct yellow colonies from MSA were streaked again

onto MSA and stored in glycerol stocks. Initial identification was done based on colony morphology. Afterwards, Gram staining and biochemical characterization were carried out for complete identification which included catalase and coagulase tests.

Antibiotic sensitivity testing of *S. aureus*

Cluster sampling was done for the selection of *S. aureus* isolated from different cities to perform antibiotic susceptibility analysis. For this, the Kirby-Bauer disk diffusion susceptibility test was used. The inoculum solution was standardized using McFarland 0.5M with Wickerham Card to achieve a comparable zone of inhibition against the selected antimicrobials (Table 1). For quality control, ATCC strains were used: E. coli (ATCC 25922) and *S. aureus* (ATCC 25923). The zones of inhibition were recorded and compared to the criteria set by the Clinical and Laboratory Standards Institute (CLSI) 2017. On this criterion, the organisms were classified as Resistant (R), Intermediate (I) or Susceptible (S).

Table 1. Brand names, generic names, symbols, and company names of antibiotics used with their potency**.**

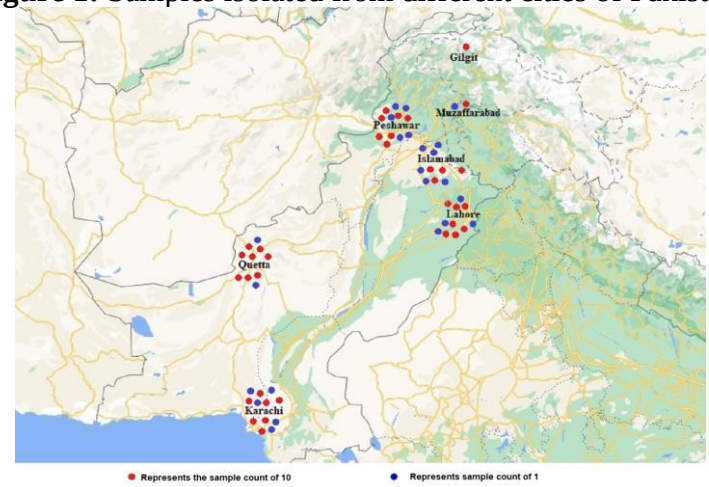
Sr. No.	Antibiotics	Class of antibiotics	Doses (µg)	Brand Names	Symbols
1.	Amikacin	Aminoglycosides	30	(Oxoid™)	AK
2.	Amoxicillin-clavulanic acid	Penicillin	30	(Liofilchem™)	AUG
3.	Ampicillin	Penicillin	10	(Oxoid™)	AMP
4.	Azithromycin	Macrolides	15	(Liofilchem™)	AZM
5.	Ampicillin-sulbactam	Penicillin	20	(Oxoid™)	SAM
6.	Cefazolin	Cephalosporin	30	(Oxoid™)	KZ
7.	Cefepime	Cephalosporin	30	(Oxoid™)	FEP
8.	Cefotaxime	Cephalosporin	30	(Liofilchem™)	CTX
9.	Cefoxitin	Cephalosporin	30	(Liofilchem™)	FOX
10.	Ceftiofur	Cephalosporin	5	(Oxoid™)	EFT
11.	Ceftazidime	Cephalosporin	30	(Liofilchem™)	CAZ
12.	Clarithromycin	Macrolides	15	(Oxoid™)	CLR
13.	Chloramphenicol	Amphenicols	30	(Liofilchem™)	C
14.	Ciprofloxacin	fluroquinolones	30	(Oxoid™)	CIP
15.	Colistin	Polymyxin	10	(Oxoid™)	CT
16.	Doxycycline	Tetracycline	30	(Oxoid™)	DO
17.	Enrofloxacin	Fluoroquinolones	5	(Oxoid™)	ENR
18.	Ertapenem	Carbapenems	10	(Liofilchem™)	ETP
19.	Erythromycin	Macrolides	15	(Liofilchem™)	E
20.	Florfenicol	Amphenicols	30	(Liofilchem™)	FFC
21.	Gentamicin	Aminoglycosides	10	(Liofilchem™)	CN
22.	Imipenem	Carbapenems	10	(Oxoid™)	IPM
23.	Linezolid	Oxazolidinones	30	(Liofilchem™)	LNZ
24.	Meropenem	Carbapenems	10	(Oxoid™)	MEM
25.	Methicillin	Penicillin	5	(Liofilchem™)	MET
26.	Minocycline	Tetracycline	30	(Oxoid™)	MH
27.	Nalidixic acid	Quinolones	30	(Oxoid™)	NA
28.	Penicillin	Penicillin	10	(Oxoid™)	P
29.	Piperacillin-Tazobactam	Penicillin	10	(Oxoid™)	TZP
30.	Rifampin	Rifamycin	5	(Oxoid™)	RD
31.	Quinopristin/Dalfopristin	Streptogramins	15	(liofilchem™)	QDA
32.	Streptomycin	Aminoglycosides	10	(Liofilchem™)	S
33.	Sulfamethoxazole+trimethoprim	Sulfonamides and Diaminopyrimidines	30	(liofilchem™)	SXT
34.	Tetracycline	Tetracycline	30	(Oxoid™)	TE
35.	Trimethoprim	Diaminopyrimidines	5	(Oxoid™)	TM

Result

Samples collection and processing

In this study, 372 samples were obtained from major cities of Pakistan (Figure1). All these samples showed growth in BPW.

Figure 1: Samples isolated from different cities of Pakistan.

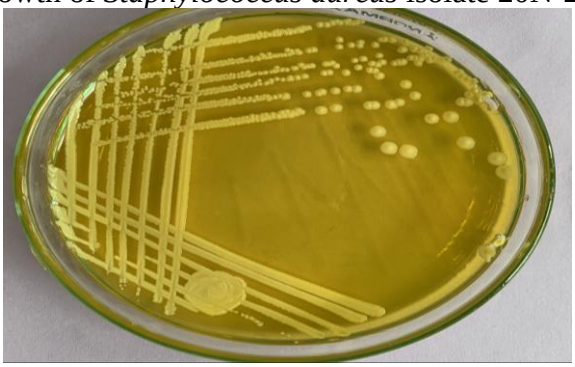


Fecal samples were collected from major cities (Gilgit, Muzaffarabad, Peshawar, Islamabad, Lahore, Quetta and Karachi) of all the province of Pakistan)

Isolation, purification, and biochemical characterization of *Staphylococcus aureus*

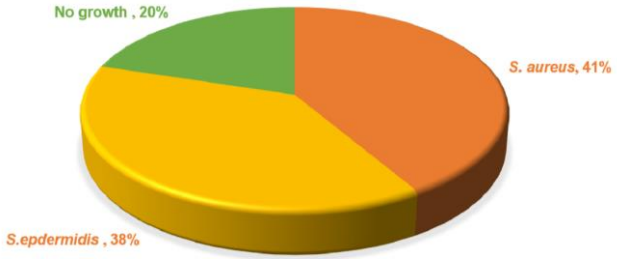
Among 372 isolates, 297 showed growth on MSA and identifed as *Staphylococcus* spp. (Figure 3). From 297 isolates, 154 isolates fermented the mannitol, resulted in the formatiom of yellow colonies on mannitol salt agar (MSA) (Figure 2) and were suspected as *S. aureus*. These isolates were gram-positive, catalase and coagulase positive which confirmed their identity of being *S. aureus*.

Figure 2**:** Growth of *Staphylococcus aureus* isolate 20N-2053 on MSA



Purification of *S. aureus* iso late 20N-2053 on MSA using quadrate streak technique. *S. aureus* ferment the mannitol salt in the MSA, changes MSA color from pink to yellow.

Figure3: Prevalence of *Staphylococcus aureus* from poultry husbandry of Pakistan.



Out of 372 samples, 75 showed no growth, 154 isolates were *S. aureus,* and 143 were *S. epidermidis.*

Antibiotic susceptibility testing of *S. aureus*

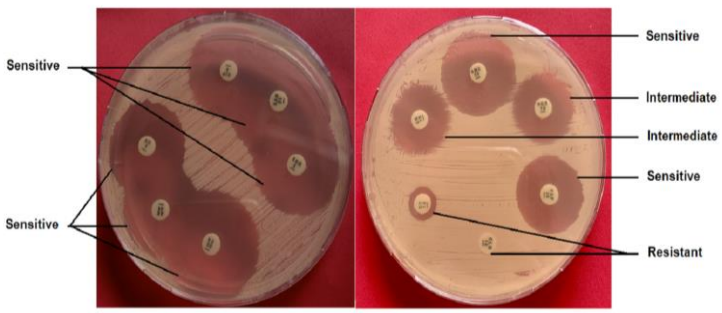
All forty-five isolates of *S. aureus* were completely sensitive to ampicillin-sulbactam, minocycline and linezolid (Table 2) while most of them were resistant to ceftazidime, nalidixic acid, erythromycin, clarithromycin, and tetracycline (Figure 4). *S. aureus* isolated from samples of Lahore and Islamabad showed similar resistant patterns but from Muzaffarabad, these isolates showed the highest resistance pattern as compared to other cities. Samples from Karachi showed the highest pattern of sensitivity of *S. aureus*, but they were completely resistant to quinolones for it, moreover, samples from Lahore and Islamabad showed complete resistance against ceftazidime and clarithromycin.

Table 2: Antibiotic susceptibility pattern and resistant percentage of selected *S. aureus* isolates against selected antibiotics based on CLSI 2020 and WHONET

Anti-biotics	% S*	% I*	%R*	Antibiotics	% S*	% I*	% R*
AK	96%	4%	0%	E	0%	13%	87%
AUG	96%	4%	0%	FFC	29%	33%	38%
AMP	82%	18%	0%	CN	89%	11%	0%
SAM	100%	0%	0%	IPM	87%	7%	7%
AZM	0%	16%	84%	LNZ	100%	0%	0%
KZ	84%	16%	0%	MEM	87%	7%	7%
FEP	78%	18%	4%	MH	100%	0%	0%
CTX	27%	27%	47%	MET	87%	9%	4%
FOX	98%	2%	0%	NA	0%	9%	91%
EFT	88%	13%	0%	P	47%	0%	53%
CAZ	0%	2%	98%	TZP	87%	13%	0%
C	29%	33%	38%	QDA	69%	18%	13%
CIP	60%	18%	22%	RD	98%	0%	2%
CLR	0%	0%	100%	SXT	89%	11%	0%
CT	49%	0%	51%	S	33%	13%	53%
DO	31%	22%	47%	TE	13%	4%	82%
ENR	62%	24%	13%	TM	84%	7%	9%
ETP	24%	42%	33%				

*S=Susceptible number of isolates, Value, *I=number of isolates with intermediate value, *R=Resistant number of isolates , Amikacin=AK, Amoxicillin Clavulanate=AUG, Ampicillin=AMP, Ampicillin-Sulbactam=SAM, Azithromycin=AZM, Cefazolin=KZ, Cefepime=FEP, Cefotaxime=CTX, Cefoxitin=FOX, Ceftiofur=EFT, Ceftazidime=CAZ, Chloramphenicol=C, Ciprofloxacin=CIP, Clarithromycin=CLR, Colistin=CT, Doxycycline=DO, Enrofloxacin=ENR, Ertapenem=ETP, Erythromycin=E, Florfenicol=FFC, Gentamicin=CN, Imipenem=IPM, Linezolid=LNZ, Meropenem=MEM, Minocycline=MH, Methicillin=MET, Nalidixic Acid=NA, Penicillin=P, Piperacillin-Tazobactam=TZP, Quinopristine/Dalfopristine=QDA, Rifampin=RD, Sulfamethoxazole-Trimethoprim=SXT, Streptomycin=S, Tetracycline=TE, Trimethoprim=TM

Figure 4: Antibiotic susceptibility pattern of isolate 20N-2288 and 20N-2169.

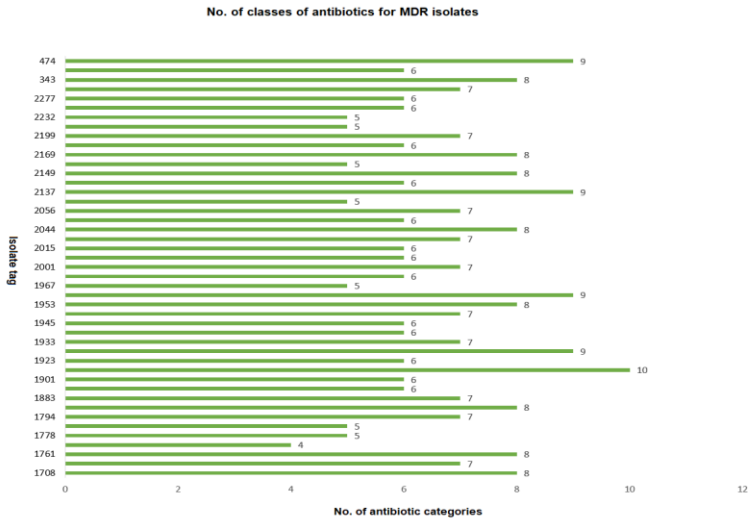


Left picture: Isolate 20N-2288 was sensitive against KZ, RO, CIP, SAM, AK and ENR. Right picture: Isolate 20N-2169 was sensitive against AMC and FEP, intermediate against CN and NOR, resistant against CT and CLR.

Drug resistance pattern of *S. aureus*

All 45 isolates of *S. aureus* were multidrug-resistant, and **most of them** were resistant to six different classes of antibiotics (Figure 5). Forty-four isolates were resistant to cephalosporins and macrolides class of antibiotics while thirty-five and thirty-seven isolates were resistant to quinolones and tetracycline, respectively.

Figure 5**:** Number of classes antibiotics of each MDR isolate of *S. aureus.*



Discussion

S. aureus is an important pathogen that is the main cause of infections in poultry e.g., pododermatitis, septicemia. Staphylococcosis caused by *S. aureus* have varying degrees of morbidity and mortality. It has been reported as a leading pathogen in the poultry industry with a 38.5 % average disease prevalence in 21 European countries [28]. Prevalence of *S. aureus* (41%) was observed in the current work and Waters *et al.,* (2011) reported the same prevalence from the fecal samples [29]. While Suleiman *et al.,* (2013) reported a higher prevalence (52%) although Ali *et al.,* (2017) showed a less prevalence (28%) [30, 12].

In this study, among all the samples of *S. aureus*, 53% of isolates showed resistance against penicillin. Nevertheless, *S. aureus* from samples of Muzaffarabad, and Gilgit were completely resistant to penicillin. These isolates were resistant to tetracycline (82%), erythromycin (87%), and penicillin (53%) and a similar trend was shown by Yin *et al.,* (2010); Agyare *et al.,* (2018)) [31, 32]. These three antibiotics are most commonly used in poultry husbandry for prophylactic, metaphylactic and therapeutic purposes hence might be responsible for the increase in resistance in *S. aureus* isolated from healthy birds [19].

Among all of these isolates only 4% were resistant to methicillin and 9% had intermediate values while 87% were sensitive to methicillin. Even though MRSA was more prevalent (19%) in the study by Ali et al., (2017) [12]. The reason behind the low prevalence of MRSA might be due to the less common use of methicillin and oxacillin for treatment and growth purposes. These isolates were slightly resistant to carbapenems, but they were quite resistant to cephalosporins, particularly ceftazidime (98%). Isolates showed intermediate and sensitive value (27%) against cefotaxime and have more chances to develop resistance against it if being used frequently. In this study resistance by these isolates to macrolides; azithromycin, clarithromycin, and erythromycin was observed as 87%, 84% and 87% respectively, might be attributed to the administration of macrolides, tylosin and kitasamycin in the form of feed additives. Amoako *et al.,* (2020) reported fewer resistant

isolates (61%) against tetracycline while in the present study, more isolates were resistant (82%) to tetracycline [2]. The cause of this resistance could be its association with the use of tetracycline analogues in poultry production. Selective pressure against this antibiotic is reported worldwide, particularly in Pakistan. Nonetheless, these Isolates were completely susceptible to minocycline which belongs to the 2nd generation of the tetracycline class because this antibiotic is not being used in the poultry industry of Pakistan. Most of the isolates (93%) were resistant to quinolones. Among quinolones, resistance against nalidixic acid by these isolates was (91%).

Seven antibiotics which are characterized as critically important by world health organization (WHO), include colistin and in this study, more than half of *S. aureus* isolates were resistant to it. This antibiotic is the last resort for the treatment of human infections. *Mcr-1* gene which governs the resistance to colistin was reported from human and broiler poultry birds in Pakistan. If the use of colistin will not be reduced, then this will pose an imminent threat to the development of more resistant microorganisms against colistin as the same case was reported in China [33].

S. aureus from Peshawar isolates showed a similar resistant pattern like Karachi. Percentage analysis of antibiotic susceptibility testing of samples obtained from Muzaffarabad showed that these isolates were completely resistant to penicillin, erythromycin, ceftazidime, nalidixic acid, ciprofloxacin, and tetracycline. There is poor legislation regarding antimicrobial use in poultry husbandry in Muzaffarabad which could be the cause of the highest level of resistance among all cities. *S. aureus* isolated from the samples obtained from Gilgit Baltistan showed a similar resistant panel of antibiotics with Muzaffarabad but overall, they were least resistant to each antibiotic of the resistant panel.

Prevalence and antibiogram of *S. aureus* are important factors to control the use of antibiotics which have the probability to be completely ineffective and should be discontinued or should be limited in use.

Conclusion

In this study, from a total of 372 samples, 154 isolates were identified as *Staphylococcus aureus* and 143 isolates of *Staphylococcus epidermidis* were identified. Antibiogram of *S. aureus* was analyzed, and overall high resistance was observed against ceftazidime, nalidixic acid, erythromycin, clarithromycin, and tetracycline with 98%, 91%, 87%, 84%, and 82% respectively and isolates were completely susceptible to ampicillin-sulbactam, linezolid and minocycline. All isolates were MDR, and they were resistant to at least 4 unique classes of antibiotics and among them, only 4% of isolates were found to be MRSA. Approximately half of the *S. aureus* isolates (51%) were resistant to colistin, a last resort antibiotic. The development of drug resistance by *S. aureus* is a matter of concern for food safety because this resistance is transferred from animals to humans. Surveillance of an antibiogram of *S. aureus* is prudent for one health concept. The development of novel antibiotics is necessary for the treatment of infections caused by multi-drug-resistant bacteria.

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References

1. Wertheim HF, Vos MC, Ott A, van Belkum A, Voss A, Kluytmans JA, van Keulen PH, Vandenbroucke-Grauls CM, Meester MH, Verbrugh HA. et al. Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. *The Lancet* 2004; 364(9435): 703-705.
2. Amoako DG, Somboro AM, Abia AL, Molechan C, Perrett K, Bester LA, Essack SY. et al. Antibiotic resistance in *Staphylococcus aureus* from poultry and poultry products in uMgungundlovu District, South Africa, using the “Farm to Fork” approach. *Microbial Drug Resistance* 2020; 26(4): 402-411.

3. Aydin A, Sudagidan M, Muratoglu K. Prevalence of staphylococcal enterotoxins, toxin genes and genetic-relatedness of foodborne Staphylococcus aureus strains isolated in the Marmara Region of Turkey. *International journal of food microbiology* 2011; 148(2): 99-106.
4. Sasidharan S, Prema B, Latha LY. Antimicrobial drug resistance of *Staphylococcus aureus* in dairy products. *Asian Pacific journal of tropical biomedicine* 2011; 1(2): 130-132.
5. Kluytmans JA. Methicillin-resistant *Staphylococcus aureus* in food products: cause for concern or case for complacency?. *Clinical microbiology and infection* 2010; 16(1): 11-15.
6. Van Den Broek IV, Van Cleef BA, Haenen A, Broens EM, Van Der Wolf PJ, Van Den Broek MJ, Huijsdens XW, Kluytmans JA, Van De Giessen AW, Tiemersma EW. et al. Methicillin-resistant Staphylococcus aureus in people living and working in pig farms. *Epidemiology & Infection* 2009; 137(5): 700-708.
7. De Boer E, Zwartkruis-Nahuis JT, Wit B, Huijsdens XW, De Neeling AJ, Bosch T, Van Oosterom RA, Vila A, Heuvelink AE. et al. Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. *International journal of food microbiology* 2009; 134(1-2): 52-56.
8. Feßler AT, Kadlec K, Hassel M, Hauschild T, Eidam C, Ehricht R, Monecke S, Schwarz S. et al. Characterization of methicillin-resistant *Staphylococcus aureus* isolates from food and food products of poultry origin in Germany. *Applied and environmental microbiology* 2011; 77(20): 7151-7157.
9. Abdalrahman LS, Stanley A, Wells H, Fakhr MK. et al. Isolation, virulence, and antimicrobial resistance of methicillin-resistant Staphylococcus aureus (MRSA) and methicillin sensitive Staphylococcus aureus (MSSA) strains from Oklahoma retail poultry meats. *International journal of environmental research and public health* 2015; 12(6): 6148-6161.
10. Crago B, Ferrato C, Drews SJ, Svenson LW, Tyrrell G, Louie M. et al. Prevalence of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in food samples associated with foodborne illness in Alberta, Canada from 2007 to 2010. *Food Microbiology* 2012; 32(1): 202-205.
11. Chuang YY, Huang YC. Livestock-associated meticillin-resistant *Staphylococcus aureus* in Asia: an emerging issue?. *International Journal of Antimicrobial Agents* 2015; 45(4): 334-340.
12. Ali Y, Islam MA, Muzahid NH, Sikder MO, Hossain MA, Marzan LW. et al. Characterization, prevalence and antibiogram study of Staphylococcus aureus in poultry. *Asian Pacific Journal of Tropical Biomedicine* 2017; 7(3): 253-256.
13. van Wamel WJ, Rooijakkers SH, Ruyken M, van Kessel KP, van Strijp JA. et al. The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on β-hemolysin-converting bacteriophages. *Journal of bacteriology* 2006; 188(4):1310-1315.
14. Malachowa N, DeLeo FR. Mobile genetic elements of *Staphylococcus aureus*. *Cellular and molecular life sciences* 2010; (18): 3057-3071.
15. Blot SI, Vandewoude KH, Hoste EA, Colardyn FA. et al. Outcome and attributable mortality in critically ill patients with bacteremia involving methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *Archives of internal medicine* 2002; 162(19): 2229-2235.
16. Centers for Disease Control and Prevention. (2019). *Antibiotic resistance threats in the United States, 2019*. US Department of Health and Human Services, Centres for Disease Control and Prevention.
17. Aarestrup FM, Agersø Y, Ahrens P, Jørgensen JC, Madsen M, Jensen LB.et al. Antimicrobial susceptibility and presence of resistance genes in staphylococci from poultry. *Veterinary microbiology* 2000; 74(4): 353-364.
18. Nemati M, Hermans K, Lipinska U, Denis O, Deplano A, Struelens M, Devriese LA, Pasmans F, Haesebrouck F. et al. Antimicrobial resistance of old and recent Staphylococcus aureus isolates from poultry: first detection of livestock-associated methicillin-resistant strain ST398. *Antimicrobial agents and chemotherapy* 2008; 52(10): 3817-3819.
19. Tanner AC. Antimicrobial drug use in poultry. Antimicrobial therapy in veterinary medicine. *Iowa State University Press, Ames, IA* 2000; 637-655.

20. Castanon JI. History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry science* 2007; 86(11): 2466-2471.
21. Dittoe DK, Ricke SC, Kiess AS. Organic acids and potential for modifying the avian gastrointestinal tract and reducing pathogens and disease. *Frontiers in veterinary science* 2018; 5: 216.
22. Economou V, Gousia P. Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infection and drug resistance* 2015; 8: 49.
23. Musawa IA, Yakubu Y, Garba B, Ballah FM, Jibril HA, Bello AS, Sani MG, Farida A. et al. Dressed chicken as potential vehicle for spread of methicillin-resistant Staphylococcus aureus in Sokoto, Nigeria. *Future Science OA* 2020; 6(10): FSO619.
24. Sadiq A, Samad M, Basharat N, Ali S, Saad Z, Khan AN, Ahmad Y, Khan A, Khan J. et al. Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Slaughter Houses and Meat Shops in Capital Territory of Pakistan During 2018–2019. *Frontiers in Microbiology* 2020; 11: 577707
25. Huyghebaert G, Ducatelle R, Van Immerseel F. An update on alternatives to antimicrobial growth promoters for broilers. *The Veterinary Journal* 2011; 187(2): 182-188.
26. Zhou W, Wang Y, Lin J. Functional cloning and characterization of antibiotic resistance genes from the chicken gut microbiome. *Applied and environmental microbiology* 2012; 78(8): 3028-3032
27. Yeoman CJ, Chia N, Yildirim S, Miller ME, Kent A, Stumpf R, Leigh SR, Nelson KE, White BA, Wilson BA. et al. Towards an evolutionary model of animal-associated microbiomes. *Entropy* 2011; 13(3): 570-594.
28. Vickers NJ. Animal communication: when i'm calling you, will you answer too?. *Current biology* 2017; 27(14): R713-R715.
29. Waters AE, Contente-Cuomo T, Buchhagen J, Liu CM, Watson L, Pearce K, Foster JT, Bowers J, Driebe EM, Engelthaler DM, Keim PS. et al. Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. *Clinical Infectious Diseases* 2011; 52(10): 1227-1230.
30. Suleiman A, Zaria LT, Grema HA, Ahmadu P. et al. Antimicrobial resistant coagulase positive *Staphylococcus aureus* from chickens in Maiduguri, Nigeria. *Sokoto Journal of Veterinary Sciences* 2013; 11(1) :51-55.
31. Yin Y, Lei F, Zhu L, Li S, Wu Z, Zhang R, Gao GF, Zhu B, Wang X. et al. Exposure of different bacterial inocula to newborn chicken affects gut microbiota development and ileum gene expression. *The ISME journal* 2010; 4(3): 367-376
32. Agyare C, Boamah VE, Zumbi CN, Osei FB. et al. Antibiotic use in poultry production and its effects on bacterial resistance. *Antimicrobial resistance—A global threat* 2018; 33-51.
33. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF. et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *The Lancet infectious diseases* 2016; 16(2): 161-168