

Detection and Antimicrobial Resistance of Staphylococcus Spp From Chicken, Litter and Humans in Addis Ababa, Ethiopia

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Abstract

Background: In veterinary medicine, three *Staphylococcus species* are of particular importance as a primary cause of specific diseases; *S. aureus* (mastitis in ruminants, equine botryomycosis and bumble foot in poultry), *S. hycus* (porcine exudative epidermitis) and *S. intermedius* (canine pyoderma). The disease conditions caused by *Staphylococcus* in poultry vary with the site, the route and predisposing factors include wounds as a result of fighting/cannibalism, immunosuppression based on virus infections or parasite infestations, and bad husbandry conditions (overcrowding). Besides their role as colonizer or pathogen in different hosts, *Staphylococcus*, which colonizes food-producing animals, can contaminate carcasses during slaughter and play a role as contaminant in the subsequent manufacturing process in food of animal origin, such as pork, beef, veal, milk, poultry meat or poultry meat products

Methods: A cross-sectional study was conducted on apparently healthy chicken, farm personnel and litter at chicken farms in Addis Ababa, Ethiopia from March 2015 to May 2015. The objectives of this study were to isolate and identify *Staphylococcus* spp from chicken, litter and personnel at chicken farm; and to determine the antimicrobial susceptibility profile of the isolates. A total of 222 samples consisting of 101 cloacal swabs, 90 tracheal swabs, 17 pooled litter swabs, 7 nasal swabs and 7 pooled hands and boot swabs were collected from six farms and examined for the presence of *Staphylococcus* species and antimicrobial resistance against 10 antimicrobial agents following recommended standard procedures.

Results: The result showed that the overall proportion of *Staphylococcus* was 64/222 (28.83%). Of the isolates 40/64 (62.5%), 11/64 (17.2%), 3/64 (4.7%) and 10/64 (15.6%), were *S. aureus*, *S. hycus*, *S. intermedius* and CNS, respectively. Only one isolate of *S. aureus* was susceptible to all antimicrobials tested. Of the 10 antibiotics tested, Penicillin G showed the highest (96.9%) resistance followed by Tetracycline (78.1%), Amoxicillin and Erythromycin at the same level (65.6%). Conversely, Ciprofloxacin showed the highest susceptibility (95.3%) followed by Sulphamethoxazole-trimethoprim (85.9%). Out of 64 isolates, 61/64 (95.3%) were resistant to three or more antimicrobials tested. Of the isolates, 38/40 (95%) *S. aureus*, 10/11 (90.9%) *S. hycus*, 3/3 (100%) *S. intermedius* and 10/10(100%) CNS showed multi drug resistance (to three or more antimicrobials).

Conclusion: This study showed considerable proportion of *Staphylococcus spp* in chicken, litter and farm workers with a potential source of resistant *Staphylococcus* species more importantly multi drug resistance strains. Further study on molecular characterization of the isolates will be essential to identify the resistant genes and establish epidemiological link in the transmission dynamics of resistant *Staphylococcus* species between poultry and humans.

Background

Staphylococci are facultative anaerobic, Gram-positive, and immotile cocci, which commonly form grape-like clusters. (Selbitz 2007; Todar, 2008). In humans and animals, many staphylococcal species are

commensals on skin and, mucosal surfaces such as upper respiratory tract, alimentary tract and genitourinary tract (Aarestrup and Schwarz, 2006; Weese, 2010). In addition to their wide distribution, staphylococci can easily spread between different animal species, and also between humans and animal species. The sources of infection are mainly contaminated foods, water and equipment, carrier and clinically infected human and animals and environment where the animals are crowded together. Various transmission routes have been described including direct, often via the hands, contact with excretions or contact with non-living objects (fomites), ingestion of contaminated food and water, aerosol and via vectors (Cuny et al., 2010; Ferreira et al., 2011)

Among the Staphylococci several studies identified the most pathogenic one LA-MRSA isolates from pig, veal calf and dairy farms and those persons with occupational contact to livestock, such as farmers, veterinarians or abattoir workers, and other persons with exposure to livestock (Köck et al. 2013). Although poultry plays a major role in intensive animal husbandry, there are only limited studies available on isolation of *Staphylococcus* from poultry and also from food of poultry origin intended for human consumption. Staphylococcal infections in both, animals and humans, are commonly treated with antimicrobial agents, most often with β -lactam antibiotics. These antibiotics were initially highly effective against staphylococci, but β -lactamase-producing *Staphylococcus* isolates emerged in the mid-1940s, and their prevalence increased dramatically within a few years. (Liu et al., 2012; Geenen et al., 2013). Staphylococci organism specially *S. aureus* strains are known to produce beta-lactamases and a modified PBP, expression of an alternative penicillin-binding protein, called PBP2a or PBP2' and acquired resistance by mobile genetic elements, in particular plasmids and transposons possibly contributing to the emergence of multiple drug resistant (McCallum et al., 2010).

In Ethiopia, investigations on bacterial isolation and identification from poultry and poultry farms in general and isolation and identification of *Staphylococcus* species in particular have received little attention. To the best of our knowledge, no significant research has been reported in the country pertaining *Staphylococcus* isolation and identification from poultry and poultry farms.

Therefore, the objectives of this study were to isolate and identify and to assess the antimicrobial susceptibility patterns of *Staphylococcus spp* from chicken, litter and poultry farms personnel in Addis Ababa.

Materials And Methods

Study Area

The study was conducted from October 2014 to May, 2015 in and around Addis Ababa. Addis Ababa is the capital city and administration centre for the Federal Democratic Republic of Ethiopia. Currently, there are 10 sub-cities "*Kifle Ketemas*" in Addis Ababa city administration delineated on the basis of geographical set up, population density, asset and service providers' distribution and convenience for administration (AACAA, 2004). It is situated at latitude of 9°3'North and 38°43' East longitudinally. It lies in

the central high lands of Ethiopia at an altitude of 2400 m.a.s.l. It has an average rainfall of 1800 mm per annum. The annual average maximum and minimum temperature is 26 °C and 11 °C, respectively; with an overall average of 18.7 °C. Highest temperatures are reached in May. The main rainy season extends from June to September. It has a relative humidity varying from 70–80% during rainy season and 40–50% during the dry season. Addis Ababa covers about 54,000 hector of land with an average population of more than 3 million (NMSA, 2007).

Study Population

The study population constituted apparently healthy chicken (i.e. broiler and layer) in chicken farms, farm environment (litter) and farm attendants.

Study Design

A cross-sectional design was used to generate the desired data.

Samples and Sampling

A total of 222 samples were collected randomly from apparently healthy exotic chicken from the farms. The sample types were cloacal and tracheal swabs from both layer and broiler, pooled litter swabs from selected farms and nasal and pooled hand and boot swabs from farm workers.

An informed verbal consent was obtained from farm owners and farm workers. Cloacal and tracheal swabs were taken from each selected poultry farms and after handling the selected chicken properly. Briefly, one person held the chicken firmly while another person inserted the swab slowly “screwing” it into the tracheal cavity or cloaca of the bird. For every swab sample, (BPW) was used. Prior to sampling, swab tips were moistened in the buffered peptone water (mainly for litter swab) and swabbed by rotating and rubbing against the sampled surface several times and kept in a sterile test tube filled with 5 ml of BPW. All samples were collected aseptically using disposable gloves to avoid contamination. Each sample was labelled with necessary information, including date of sampling, type of sample, source of sample (farm) and identification of the animal labelled with permanent marker. At all levels of sampling, the samples were placed in the rack for easy handling and held in an ice box, properly packed and kept cold. Finally it was transported to the Microbiology laboratory at the College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu for bacteriological analysis

Isolation and Identification

The International Organization for Standardization, ISO 6888-3:2003 was employed for the isolation and identification of *Staphylococcus* species from swab samples. The swabbed samples which were kept in a tube containing BPW were put overnight in a refrigerator. A loop full of the pre-enriched samples various dilutions were streaked on blood agar plate (BAP) enriched with 7% heparinised sheep blood and incubated at 37°C for 24–48 hours under aerobic conditions. The plates were examined for the presence of *Staphylococcus* colonies based on the morphological characteristics (creamy, greyish, white or yellow

colonies) and haemolytic pattern. The presumed colonies were further sub-cultured on Nutrient agar Plate (NAP) and incubated at 37°C for 24–48 hours to obtain pure colonies). The pure colonies were transferred to nutrient slants and stored at 4°C for further biochemical and antimicrobial susceptibility tests.

Identification of staphylococci organisms and species assignment were done based on KOH test, gram's staining, catalase test, oxidation and fermentation test, sugar fermentation (mannitol and maltose) tests and coagulase test.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility pattern of the isolates were determined against 10 antimicrobial agents using the Kirby-Bauer disc diffusion method. Briefly, sterile cotton swab was used to streak the bacterial colonies on the surface of Mueller Hinton agar plates and were allowed to dry for 15 minutes before applying the discs. Filter paper disks containing designated amount of the impregnated antimicrobial drugs obtained from commercial supply firms were gently and firmly placed on the agar plates using sterile toothed forceps. All the discs were gently pressed with forceps to insure complete contact with the agar surface. The discs were placed 1.5 cm away from the edge of the plates and they were 3 cm apart from each other. The plates were then incubated inverted aerobically at 37 °C for 18–24 hours. The diameter of the inhibition zones around disk were measured using calliper on the back of the plates and the isolates were interpreted as susceptible, intermediate and resistant according to guidelines of (CLSI, 2013).

Data Management And Analysis

The generated data were stored in Microsoft excel and analysed using SPSS 20 version software. Descriptive statistics such as percentage, proportion and frequency distributions were applied to compute some of the data. The Pearson's chi-square (χ^2) test was used to assess the differences of proportion of *Staphylococcus* and *Staphylococcus* species among the sample sources and types. The difference was considered statistically significant when the *P*-value was less than 0.05.

Results

Proportional distribution of Staphylococcus isolates in farm

Of an overall examined samples from six different farms (222), 64 (28.8%), [95%CL:28.12–29.54] were positive for *Staphylococcus* species, from which 4/22 (18.2%), 21/59 (35.6%), 10/28 (35.7%), 14/62 (22.6%), 11/23 (47.8%) and 4/28 (14.3%) from AM, BD, EU, HM, SA, and TE farm, were positive for *Staphylococcus* species, respectively. There was statistically differed significantly in isolation of *Staphylococcus* between different farm ($p = 0.046$)

Proportional distribution of Staphylococcus isolates in housing system and sample source

From two poultry housing system, cage type and deep litter system, 4/28 (15.69%), and 58/194 (31.71%), were positive for *Staphylococcus*, respectively. For samples examined from layers (Bo vans Brown) and broilers (White Leg Horne), 10/57 (17.54%) and 45/134 (33.58%) were positive for *Staphylococcus*, respectively, but the highest percentage 7/17 (41.18%) was isolated from litter and the lowest percentage 2/4 (14.29%) was isolated from farm workers, however there was no statistically significant difference in isolation of *Staphylococcus* between different housing system (P-value = 0.103) and sample source (P-value = 0.052) (Table 1).

Proportional distribution of *Staphylococcus* isolates in sample type

Of the total 64/222 (28.8%) *Staphylococcus* isolates, 33/101 (32.7%), 22/90 (24.44%), 7/17 (41.2%) and 2/7 (28.6%), from cloacal swab, tracheal swab, pooled litter swab and nasal swab of farm attendants, were positive for *Staphylococcus*, respectively, but no isolate was found from pooled hand and boot swab of farm attendants 0/7 (0%). There was no statistically significant difference in *Staphylococcus* isolation between different sample type (P-value = 0.225) (Table 1).

Proportional distribution of *Staphylococcus* species in farm

Table 1

Distribution of *Staphylococcus* isolates in different farms, housing system, sample source and sample types

		Positive	Total	Prevalence (%)	95%CI	χ^2	P
Farm name	M	4	22	18.18	16.40, 19.96	11.92	0.046
	D	21	59	35.59	34.07, 37.12		
	U	10	28	35.71	33.50, 37.93		
	M	14	62	22.58	21.40, 23.76		
	A	11	23	47.83	45.00, 50.65		
	E	4	28	14.29	12.89, 15.69		
Housing system	C	4	28	14.29	12.89, 15.69	3.30	0.07
	L	60	194	30.93	30.15, 31.71		
Sample source	B	45	134	33.58	32.60, 34.56	7.72	0.052
	L*	7	17	41.18	38.13, 44.23		
	Ly	10	57	17.54	16.46, 18.63		
	P	2	14	14.29	12.31, 16.27		
Sample type	C	33	101	32.67	31.56, 33.79	5.67	0.225
	HB	0	7	0.00	-		
	L*	7	17	41.18	38.13, 44.23		
	N	2	7	28.57	24.61, 32.53		
	T	22	90	24.44	23.42, 25.47		
Total		64	222	28.83	28.12, 29.54		

Key = AM = Amelewerk farm enterprise; BD = Bayissa Damessa farm; EU = Europe farm enterprise; HM = Haile Michael farm; SA = Senait and Abdella and their friends TE = Tesfaye farm enterprise; C = Cage type housing system; L = Litter type housing system; P = Personnel, Ly = layer, B = Broiler, Cl = Cloacal swab; L* = Litter swab, T = Tracheal swab; N = Nasal swab; HB = Hand

Proportional distribution of *Staphylococcus* species in housing system

After isolation and identification of *Staphylococcus* species, 40 (18.01%), 11 (4.95%), 3 (1.4%), 10 (4.5%) were *S. aureus*, *S. hycus*, *S. intermedius* and Coagulase negative staphylococci (CNS) from the total sample examined, respectively and none of the farm is free of the bacteria. Of the isolates, *S. aureus*

40/64 (62.5%) was the most dominant followed by *S. hycus* 11/64 (17.2%) and CNS 10/64 (15.6%); and lastly *S. intermedius* 3/64 (4.7%). From the species isolated and identified only *S. aureus* was isolated from all farms with highest prevalence in farm BD, 19/59 (32.2%), and lowest in farm SA, 1/23 (3.4%). Other staphylococcal species, *S. hycus* was isolated only from three farms, BD 2/59 (3.4%), EU 3/28 (10.7%) and SA 6/23 (26.1%); and *S. intermedius* and CNS were also isolated from three similar farms, EU, HM and SA, with 1/28 (3.6%), 1/62 (1.6%), 1/23 (4.3%) *S. intermedius* and 4/28 (14.3%), 3/62 (4.8%) and 3/23 (13%) CNS, respectively. There was statistically differed significantly in isolation and identification of *Staphylococcus* species between different farm except for *S. intermidius* (P = 0.53) (Table 2).

Proportional distribution of *Staphylococcus* species in sample source and sample type

From two types of housing system (cage and deep litter) 34/194 (18.6%) and 4/28 (14.3%) from deep litter and cage type housing system, were positive of *S. aureus*, respectively, but *S. hycus*, 11/194 (5.7%), *S. intermedius*, 3/194 (1.6%) and CNS, 10/194 (5.2%) were only isolated from deep litter housing system, and there was no statistically significant difference in *Staphylococcus* species isolated and identified between housing system, since the P-value in all species is (P-value > 0.05) (Table 2).

Among the *Staphylococcus* species *S. aureus* was isolated from all sample sources, broiler (17.9%), layer (17.5%), litter (23.5%) and personnel (14.3%). Both *S. hycus* and CNS with the same result (6.7%) were isolated from broilers; *S. intermedius* was only isolated from broiler (2.2%). There was no statistically significant difference in *Staphylococcus* species isolated and identified between different sample sources (P-value \geq 0.05 in all species) (Table 2). All the staphylococcal species were isolated from different sample types, but none of them identified from pooled hand and boot swab of farm attendants. *S. aureus* was highest (28.6%) in nasal swab and lowest (12.9%) in cloacal swab. *S. aureus* 4(23.5%). *S. hycus* 2(21.8%) and CNS 1(5.9%) were isolated from litter, but *S. intermidius* not. *S. intermidius* was only isolated from cloacal swab, 3/101 (3%). There was no statistically significant difference in *Staphylococcus* species isolated from different sample types except CNS (p = 0.05) (Table 2).

Table 2

The proportional distribution of *Staphylococcus* species isolated from different farms, housing systems, sample sources and different sample types

		<i>Staphylococcus</i> species, n (%)			
		CNS	SA	SH	SI
Farm name	AM (n = 22)	0 (0.0)	4 (18.2)	0 (0.0)	0 (0.0)
	BD (n = 59)	0 (0.0)	19 (32.2)	2 (3.4)	0 (0.00)
	EU (n = 28)	4 (14.3)	2 (7.1)	3 (10.7)	1 (3.6)
	HM (n = 62)	3 (4.8)	10 (16.1)	0 (0.0)	1 (1.6)
	SA (n = 23)	3 (13)	1 (3.4)	6 (26.1)	1 (4.3)
	TE (n = 28)	0 (0.0)	4 (14.3)	0 (0.0)	0 (0.0)
χ^2 (p-v)		15.28(0.01)	13.60(0.02)	29.93(0.00)	4.12(0.53)
Housing system	C (n = 28)	0 (0.0)	4 (14.3)	0 (0.0)	0 (0.00)
	L (n = 194)	10 (5.2)	36 (18.6)	11 (5.7)	3 (1.6)
χ^2 (p-v)		2.30(0.32)	0.32(0.88)	2.55(0.29)	0.67(0.72)
Sample source	B (n = 134)	9 (6.7)	24 (17.9)	9 (6.7)	3 (2.2)
	L* (n = 17)	1 (5.9)	4 (23.5)	2 (11.8)	0 (0.0)
	Ly (n = 57)	0 (0.0)	10 (17.5)	0 (0.00)	0 (0.0)
	P (n = 14)	0 (0.0)	2 (14.3)	0 (0.0)	0 (0.0)
χ^2 (p-v)		4.95(0.18)	0.50(0.92)	6.26(0.10)	2.00(0.57)
Sample type	C (n = 101)	9 (8.9)	13 (12.9)	8 (7.9)	3 (3)
	HB (n = 7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	L* (n = 17)	1 (5.9)	4 (23.5)	2 (11.8)	0 (0.0)
	N (n = 7)	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)
	T (n = 90)	0 (0.0)	21 (23.3)	1 (1.1)	0 (0.0)
χ^2 (p-v)		9.54(0.05)	5.95(0.20)	7.11(0.13)	3.64(0.46)
Total n = 64		10(15.6)	40 (62.5)	11(17.2)	3 (4.7)

Key = CNS = Coagulase negative *Staphylococcus*; SA = *S. aureus*; SH = *S. hycus*; SI = *S. intermedius*; and see the previous table for others

Frequency distribution of antimicrobial susceptibility pattern of Staphylococcal isolates

All isolates (64) of *Staphylococcus* were tested for susceptibility test to 10 antimicrobial discs. The comparative efficacies of antimicrobial used indicate CIP and SXT were the most effective antibiotics with susceptibility percentage of 95.3% and 85.9% respectively. Conversely P and TE have shown the poorest efficacy (susceptibility) or high resistance against staphylococcal isolates with 96.9% and 78.1%, respectively (Table 3).

Table 3
Antimicrobial susceptibility profile of staphylococcal isolates (n = 64)

S/n	Antimicrobials	Number of tested	Susceptible [N (%)]	Intermediate [n (%)]	Resistance [N (%)]
1	P	64	2 (3.1)	0 (0.0)	62 (96.9)
2	VA	64	26 (40.6)	0 (0.0)	38 (59.4)
3	CIP	64	61 (95.3)	0 (0.0)	3 (4.7)
4	SXT	64	55 (85.90)	0 (0.0)	9 (14.1)
5	AML	64	22 (34.4)	0 (0.0)	42 (65.6)
6	F	64	19 (29.70)	27 (42.2)	18 (28.1)
7	E	64	6 (9.4)	16 (25.0)	42 (65.6)
8	TE	64	7 (10.9)	7 (10.9)	50 (78.1)
9	S	64	24 (37.5)	16 (25.0)	24 (37.5)
10	NA	64	48 (75.0)	5 (7.80)	11 (17.2)
Total	10	640	270 (42.2)	71 (11.1)	299 (46.7)
Key for Abbreviations: P = Penicillin, AML = Amoxicillin, F = Cefoxitin, CIP = Ciprofloxacin, NA = Nalidixic acid, S = Streptomycin, SXT = Sulfamethoxazole trimethoprim, VA = Vancomycin, TE = tetracycline, E = Erythromycin					

Frequency distribution of antimicrobial susceptibility pattern of Staphylococcus species

Staphylococcal species have variable susceptibility pattern toward antimicrobials (Table 3). All positive samples of *Staphylococcus* species, *S. aureus* (40), *S. hycus* (11), *S. intermedius* (3) and CNS (10) were tested for susceptibility. Of the isolates 38/40 (95%) *S. aureus*, 10/11 (90.9) *S. hycus*, 10/10 (100%) CNS and 3/3 (100%) *S. intermedius* were resistance to three or more antimicrobials, while 1/40 (2.5%) *S. aureus* and 1/11 (9.1%) *S. hycus* showed mono resistance and only 1/40 (2.5%) *S. aureus* was susceptible to all antimicrobials tested. *S. aureus* is highly resistance (95.0%) to P and (72.5%) to both VA and TE; and highly susceptible (97.5%), and (87.5%) to CIP and SXT, respectively. *S. hycus* showed greater resistance (100%) to P and highly resistance (81.8%) to TE; and greater susceptible (100%) to SXT and

highly susceptible (90.9%) to CIP and slightly susceptible (63.6%) to both VA and NA. Similarly *S. intermedius* showed greater resistance (100%) to P, AML, TE and S, but greater susceptibility (100%) was seen in VA. Moreover, CNS has showed greater resistance (100%) to P and greater susceptibility (100%) to CIP (Table 4).

Table 4
Antimicrobial susceptibility patterns of *Staphylococcus* species

Antimicrobials tested		Staphylococcus species [n (%)]					x ²	P
		CNS	SA	SH	SI			
P	R(n = 62)	10 (100)	38 (95.0)	11 (100)	3 (100)	1.239	0.744	
	S (n = 2)	0 (0.0)	2 (5.0)	0 (0.0)	0 (0.0)			
VA	R(n = 38)	5 (50.0)	29 (72.5)	4 (36.4)	0 (0.0)	10.02	0.018	
	S(n = 26)	5 (50.0)	11 (27.5)	7(63.6)	3 (100)			
CIP	R (n = 3)	0 (0.0)	1 (2.5)	1 (9.1)	1 (33.3)	6.908	0.075	
	S(n = 62)	10 (100)	39 (97.5)	10(90.9)	2 (66.7)			
SXT	R(n = 9)	2 (20.0)	5 (12.5)	0 (0.0)	2 (66.7)	9.042	0.029	
	S (n = 55)	8 (80.0)	35 (87.5)	11 (100)	1 (33.3)			
AML	R(n = 42)	8 (80.0)	26 (65.0)	5 (45.5)	3 (100)	4.478	0.214	
	S(n = 22)	2 (20.0)	14 (35.0)	6 (54.5)	0 (0.0)			
F	I (n = 27)	1 (10.0)	21 (52.5)	5 (45.5)	0 (0.0)	16.032	0.014	
	R(n = 18)	7 (70.0)	6 (15.0)	4 (36.4)	1 (33.3)			
	S (n = 19)	2 (20.0)	13 (32.5)	2 (18.2)	2 (66.7)			
E	I (n = 16)	1 (10.0)	0 (0.0)	0 (0.0)	1 (33.3)	20.119	0.003	
	R(n = 42)	9 (90.0)	25 (62.5)	7 (63.6)	1 (33.3)			
	S(n = 6)	0 (0.0)	1 (2.5)	4 (36.4)	1 (33.3)			
TE	I (n = 7)	0 (0.0)	7 (17.5)	0 (0.0)	0 (0.0)	5.642	0.465	
	R(n = 50)	9 (90.0)	29 (72.5)	9 (81.8)	3 (100)			
	S(n = 7)	1 (10.0)	4 (10.0)	2 (18.2)	0 (0.0)			
S	I (n = 16)	2 (20.0)	11 (27.5)	3 (27.3)	0 (0.0)	7.27	0.297	
	R(n = 24)	4 (40.0)	15 (37.5)	2 (18.2)	3 (100)			
	S (n = 24)	4 (40.0)	14 (35.0)	6 (54.5)	0 (0.0)			
NA	I (n = 5)	0 (0.0)	4 (10.0)	1 (9.1)	0 (0.0)	4.931	0.553	
	R(n = 11)	3 (30.0)	4 (10.0)	3 (27.3)	1 (33.3)			

I = intermediate, R = resistant, S = susceptible

Antimicrobials tested	<i>Staphylococcus</i> species [n (%)]					χ ²	P
	CNS	SA	SH	SI			
S(n = 48)	7 (70.0)	32 (80.0)	7 (63.6)	2 (66.7)			
Total	10 (15.6)	40 (62.5)	11(17.2)	3 (4.7)			
I = intermediate, R = resistant, S = susceptible							

Frequency distribution of Mono and Multi-drug resistance of *Staphylococcus* species

Out of 64 *Staphylococcal* isolates, only one (1.56%) isolate of *Staphylococcus* (*S. aureus*) was susceptible to all antimicrobial agents tested, while two (3.13%) isolates of *Staphylococcus* were mono drug resistant. Multi-drug resistance to three or more antimicrobials was observed in 61 (95.31%) of all the isolates (Table 5).

Table 5
Number and percentages of antimicrobial resistance patterns of *Staphylococcus* species

No of antimicrobials	Antimicrobial resistance pattern	Number (%)	Species
One	NA	1 (1.56)	SH
	P	1 (1.56)	SA
Three	P, E, S(1); P, AML, TE (1); P, E, TE(1); P, VA, TE (2); P, F, E (1); P, VA, E (1)	7(10.94)	SA
	P, AML, TE(1) P, E, TE(1)	2 (3.13)	SH
Four	P, AML, E, TE (2); P, CIP, AML, NA (1) P, E, TE, NA (1)	4 (6.25)	SH
	P, AML, F, TE (1); P, AML, E, S (1)	2 (3.13)	CNS
	P, AML, F, TE (1) ; P, VA, AML, TE (2); P, VA, AML, E (5); P, VA, E, S (1); P, VA, TE, S (1) P, E, TE, S (1)	11(17.19)	SA
Five	P, CIP, AML, TE, E (1)	1 (1.56)	SI
	P, VA, F, E, TE (2);	2 (3.13)	SH
	P, AML, F, E, TE (1); P, VA, AML, E, TE(1); P, VA, E, TE, NA (1)	3 (4.69)	CNS
	P, AML, F, E, TE (1); P, VA, AML, E, TE(4); P, VA, SXT, AML, TE (1); P, VA, AML, TE, S (2); P, VA, TE, S, NA (1); P, VA, E, TE, S (1); P, AML, F, TE, S (1)	11 (17.19)	SA
Six	P, SXT, AML, E, TE, S (1);	1 (1.56)	SI
	P, VA, F, E, TE, S (1);	1 (1.56)	SH
	P, VA, AML, F, E, TE (1); P, AML, F, E, TE, NA (1)	2 (3.13)	CNS
	P, SXT, AML, E, TE, S (1); P, VA, AML, F, E, TE (1); P, VA, SXT, E, TE, S (1); P, VA, SXY, AML, TE, S (1); P, VA, AML, E, TE, S (2)	6 (9.380)	SA
Seven	P, SXT, AML, F, TE, S, NA (1);	1 (1.56)	SI
	P, VA, AML, F, TE, S, NA (1);	1 (1.56)	SH
	P, SXT, AML, F, E, TE, S (1) P, VA, AML, F, E, TE, S (1);	2 (3.13)	CNS
	P, VA, AML, F, E, TE, S (1); P, VA, SXT, AML, E, TE, NA (1); P, VA, CIP, AML, E, TE, NA (1);	3 (4.69)	SA
Eight	P, VA, SXT, F, E, TE, S, NA(1)	1 (1.56)	CNS

Key: MDR = Multi drug resistance, SA = *S. aureus*, SH = *S. hycus*, SI = *S. intermedius*

No of antimicrobials	Antimicrobial resistance pattern	Number (%)	Species
Total	Mono resistance (2)	63 (98.44)	SA(39)
	Multi resistance (61)		SH(11)
			SI(3)
			CNS(10)
MDR	61	61 (95.31)	SA(38)
			SH(10)
			SI(3)
			CNS(10)
Key: MDR = Multi drug resistance, SA = <i>S. aureus</i> , SH = <i>S. hycus</i> , SI = <i>S. intermedius</i>			

Discussion

Staphylococcus spp. are significant bacteria in the aetiology of avian diseases but little is known about the bacterial presence in the poultry environment such as in poultry litter and in the poultry house air as reported by Saleh *et al.* (2003). The modern poultry industry can produce market-ready broiler chickens in less than six weeks. This accomplishment has been achieved through genetic selection, improved feeding and keen health management practices including usage of antibiotics as therapeutic agents to treat bacterial diseases in intensive farming systems. Resistance against frequently used antibiotics has been observed in bacteria present in poultry since the introduction of these antimicrobial agents in poultry according to the report of Apata (2009).

In the present study, 222 samples originated from six different farms were examined. The isolation and identification results proved the presence of the *Staphylococcus* in farm originated of examined samples in the study area. The present study result showed that out of 222 samples that were taken from six different farms, 64 (28.8%) were positive for *Staphylococcus* species. This result was much lower than the finding of Modestas *et al.* (2014) who reported 71% Staphylococcal species from poultry products in Kaunas, Lithuania; but higher than 15.2% Staphylococcal species reported by Masdoq *et al.* (2008) from pathogenic bacteria associated with respiratory disease of poultry in Nigeria; and similarly Rany Roy *et al* (2012) reports 20% of *Staphylococcus* species which was slightly lower than that of the present finding. Out of 222 samples, 18.01%, 4.95%, 1.35% and 4.5% of *S. aureus*, *S. hycus*, *S. intermedius* and CNS were isolated, respectively. Other findings was reported by (El- Jake *et al* 2008) with 8%, 2% and 0% of *S. aureus*, *S. intermedius* and *S. hycus*, respectively which were lower than the present findings. The prevalence of *Staphylococcus* between farm levels showed slightly high percentage of variation in distribution, and the significance difference ($P < 0.05$) between the farms These variations might be due to difference in sample size, geographical location and type of bird; and it could also be related with poor

hygienic and sanitation status of the difference of the farms. The water and feed troughs were not properly washed and create favourable environment for the growth and proliferation of bacterial organisms when subsequently refilled without cleaning as reported by Rany Roy *et al.* (2012).

Based on the *Staphylococcus* species isolated, the result showed that *S. aureus* 40 (62.5%) was the most predominant isolate followed by *S. hycus* 11 (17.5%), Coagulase negative staphylococci (CNS) 10 (15.6%) and *S. intermedius* 3 (4.7%). The findings of the present study 40/64 (62.5%) of *S. aureus* revealed a high proportion rate than the previous findings by (Amare *et al.* (2013) who isolate 40 (23.53%) *S. aureus* out of 170 different bacterial strains isolated belonging to different genera from yolk sac infection (omphalitis) in Kombolcha poultry farm, Ethiopia and Dashe *et al.* (2013) who reported 20.5% *S. aureus* from chicken in Jos, Nigeria. Additionally, these present findings of *S. aureus* (62.15%) was slightly higher than 40%; *S. hycus* (17.5%) was higher than 4% ; *S. intermedius* (3.4%) was nearly the same with 2%; and finally CNS (15.6%) was much lower than 54% which were (i.e. 40%, 4%, 2% and 54%) the findings of Bendahou *et al.* (2008) in Iben and Jben, North Morocco. Other observation was also reported by Jakee *et al.* (2013) with a prevalence of 46% *S. aureus* from poultry products in Egypt which was less than the present study value.

In the current study all samples were collected from cage type and deep litter poultry management systems including the farm workers in each management system, and from those two management systems, 60 (30.93%) and 4 (14.29%) from deep litter and cage type, were positive for *Staphylococcus*, respectively. Regarding to the species, *S. aureus* was isolated from both cage type and deep litter type of sampled birds with the higher prevalence (18.6%) was recorded from litter type and lower prevalence (14.3%) was observed from cage type birds. But all of the other three species, *S. hycus*, *S. intermedius*, and CNS, 5.7%, 1.6% and 5.2% were isolated only from deep litter birds, respectively, and the reason may be due to infrequent cleaning in deep litter type as reported by Rany Roy *et al.* (2012).

In this earlier study, the examined samples were originated from different sources for isolation and identification of the *Staphylococcus* species. From these, 45 (33.58%), 10 (17.54%), 7 (41.18%) and 2 (14.29%) from broiler, layer, litter and farm attendants, were positive for *Staphylococcus*, respectively. The observation was recorded by Sunday *et al.* (2010) the prevalence of *Staphylococcus*, 32% from broiler which is almost nearest to the present finding (33.58%) from broilers; and similarly he also recorded 35% of *Staphylococcus* from layers which is double of the present result observed from layers (17.54%). Based on species, it is notable that all sample sources were positive of *S. aureus* with the highest value (23.5%) isolated from litter and the lowest value (14.3%) isolated from farm workers. *S. hycus* was only isolated from broiler with a result of 6.7% and from litter with a result 11.8%. Similarly, CNS was also isolated from broiler with a result of 6.7% and litter with a result of 1.9%, but *S. intermedius* was only recorded from broiler with a value of 2.2%. In this study, the *S. aureus* isolated from broiler and layer was 17.9% and 17.5%, respectively, which was nearly the same. But this finding was much lower than the finding of Rasheed (2011) who reported 50.89% *S. aureus* from broiler that cause arthritis in broiler chicken farms in Iraq. Similarly, the present finding of *S. aureus* from litter (23.5%) was lower than the

finding reported by Mohammed *et al.* (2013) 8.33% of *S. aureus* from litter swab of broiler chicken farm in Khartoum state.

On the basis of individual sample types (cloacal swab, tracheal swab, nasal swab, pooled litter swab and pooled hand and boot swab) were used for isolation and identification of *Staphylococcus* and Staphylococcal species in the current study. The proportional distribution of *Staphylococcus* from sample types is 33(32.67%), 22 (24.44%), 7 (41.18%) and 2 (28.57%) from cloacal swab, tracheal swab, pooled litter swab and nasal swab, were positive for *Staphylococcus* species, respectively. From the isolates, CNS was isolated only from cloacal swab (8.9%) and pooled litter swab (5.9%); *S. hycus* was isolated from pooled litter swab (11.8%), cloacal swab (7.9%) and tracheal swab (1.1%); *S. intermidius* was only isolated from cloacal swab (3%). *S. aureus* was almost isolated from all sample types except pooled hand and boot swab of farm workers. Its prevalence was almost similar in pooled litter swab (23.5%) and tracheal swab (23.3%) but vary in others which is highest (28.6%) in nasal swab of farm workers and lowest (12.9%) in cloacal swab. But the present finding from nasal swab (28.8%) is higher than the finding observed by Neela *et al.* (2013) who reported 3.61% *S. aureus* from nasal swab of poultry farm worker in Iran, and the present result of *S. aureus* from tracheal swab (23.3%) was much lower than the finding of Suleiman *et al.* (2013) who proved 85% of *S. aureus* from tracheal swab.

In the current study 64 staphylococcal isolates were tested using 10 antimicrobials. From these antimicrobials, Penicillin G shows the highest resistance 96.9% followed by Tetracycline, 78.1%. From *Staphylococcus* species, the highest resistance was registered for penicillin G by *S. aureus* (95.0%), which is almost similar to 96.7% *S. aureus* resistance reported by Mekuria *et al.* (2013), but slightly less than 100% resistance *S. aureus* reported by Otalú *et al.* (2011). From this study result both *S. hycus* and CNS showed the highest resistance, 100% against Penicillin G. This finding is consistent with that of Bendahou *et al.* (2008) who reports similar result of resistance from these strains obtained from milk and whey in Morocco. Thus, the results indicate that the majority of antimicrobial resistance in *S. aureus* and CNS isolates could be due to production of β -lactamases and may carry the *mecA* chromosomal gene responsible for production of the altered penicillin binding protein PBP-2a as suggested by Mamza *et al.* (2010).

Of the total isolates (64) subjected to antimicrobial susceptibility test, 61/64 (95.3%) have developed multidrug resistance (resistant to three and more than three antimicrobials). High prevalence of multi drug resistance among isolates in the present study clearly indicated the excessive or inappropriate use of antibiotics. This may be connected to the fact that antimicrobial use in commercial poultry settings is more likely to be regulated than in small holder farms. In addition, veterinarians and poultry farmers generally use these antimicrobials as prophylaxes, growth promoters or inaccurate dosages given to sick flocks by unqualified personnel may likely result high level of resistance that was reported by Suleiman *et al.* (2013) done on antimicrobial resistant *Staphylococcus* from chickens in Maiduguri, Nigeria, and it is in agreement with present finding of multi drug resistance.

Conclusion

The result of the current study showed that *Staphylococcus* is one of the organisms that widely distributed in poultry farm in the study area. In this study a total of 222 samples were examined and processed and 64 (28.83%) *Staphylococci* were isolated and of which *S. aureus* was the most dominant one, 40 (62.5%), even though little or no comparative report in Ethiopia at all. The antimicrobial susceptibility test of the *Staphylococcus* isolates in this study showed that 61/64 (95.31%) isolates, almost all were multi-drug resistance (three and more than three antimicrobials tested) and this makes an alarming cause for further study. Therefore, further studies should be conducted on large scale to find the association between source of infection and prevalence to find out the possible source of contamination with *Staphylococcus* species and antimicrobial susceptibility test should be carried out at regular intervals to find out the development of resistance against the most commonly applied antibiotics.

Abbreviations

BPW: Buffered peptone water; MDR: Multiple drug resistance; ISO: The International Organization for Standardization, BAP: blood agar plate, NAP: Nutrient agar Plate

Declarations

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Availability of data and materials

Data is not available for online access; however, readers who wish to gain access to the data can write to the corresponding author Dr Fufa Abunna at fufa.abunna@aau.edu.et

Authors' contributions

Fufa Abunna, conceived and designed the experiments; contributed reagents, materials, analysis tools or data and wrote the paper.

Biyansa Adugna, performed the experiments; analyzed and interpreted the data; wrote the paper.

Hika Waktole: Performed the experiments.

Takele B. Tufa, conceived and designed the experiments; performed the experiments; contributed reagents, materials, analysis tools or data.

Dinka Ayana: Conceived and designed the experiments

Fanta D. Gutema, Wrote and edited the paper

Reta D. Abdi: Conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data;

Ethics approval and consent to participate

The study was approved by the Ethical Clearance Committee of the college of veterinary medicine and agriculture, Addis Ababa University

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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