Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 16 Issue 2 Version 1.0 Year 2016
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Drug Resistance Pattern of *Staphylococcus* in Poultry in Central and Southern Ethiopia

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Abstract- This study was conducted from November 2014 - May 2015 to determine the antimicrobial resistance pattern of Staphylococcus species in poultry in Central and Southern Ethiopia. 205 staphylococcal species isolated from poultry were evaluated using disk diffusion method for their antimicrobial susceptibility to 10 different antimicrobial drugs. Staphylococcus were found to be highly susceptible to Ciprofloxacin (85.4%) followed by Sulfamethoxazole-Trimethoprim (68.8%). However these isolates were highly resistant to Penicillin G (94.1%) and Tetracycline (79%) followed by Amoxicillin (60.5%). From all Staphylococci isolates tested for drug susceptibility pattern, only 1 isolate (S. aureus) was susceptible to all tested drugs and 99.51% of isolates were resistant to at least one of the antibiotics tested. Coagulase negative Staphylococci were highly resistant to all tested drugs except Ciprofloxacin (0%) and S. aureus were highly resistant to Penicillin G (92.2%) and Tetracycline (74.5%). Staphylococcus species isolated in poultry in Central and Southern Ethiopia were all multidrug resistant.

Keywords: *drug resistance, ethiopia, poultry, staphylo-coccus.*

GJSFR-D Classification : *FOR Code: 830309*

Strictly as per the compliance and regulations of :

Drug Resistance Pattern of *Staphylococcus* in Poultry in Central and Southern Ethiopia

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Keywords: drug resistance, ethiopia, poultry, staphylococcus.

I. Introduction

Staphylococci are considered to be of the most common causes of infections in birds. Most infections are caused by coagulase positive *Staphylococci*, especially *Staphylococcus aureus*, but also coagulase negative *Staphylococci* seem to be associated with infections (Suleiman *et al*., 2013). The *Staphylococci* are ubiquitous in nature, with humans and animals as the primary reservoirs. It is commonly found in poultry house environment and can be isolated from the litter, dust and feathers. The bacterium is considered to be a normal resident of the chicken, located on the skin and feathers and in the respiratory and intestinal tracts. A *staphylococcus* infection, or *Staphylococcosis*, refers to a variety of diseases in poultry caused by *staphylococci* bacteria (Jensen and Miller, 2001).

The emergence of antibacterial resistance among pathogens that affect animal health is of growing concern in veterinary medicine as these resistant pathogens in animals have been incriminated as a potential health risk for humans (Moon *et al*., 2007). The rise of drug-resistant virulent strains of *Staphylococci* is a serious problem in the treatment and control of staphylococcal infections both in humans and animals. Staphylococcal infection is now a major public health problem and the poultry meat has been implicated as a main source of infection in humans (Duran *et al*., 2012).

Staphylococcus is now a serious problem worldwide due to its ubiquitous nature and the existence of highly antibiotic resistant isolates. Thus the objective of this study was to evaluate the drug resistance pattern of *Staphylococcus* isolated from poultry in central and southern Ethiopia.

II. Methodology

Staphylococcus species were isolated from poultry from Central (Bishoftu, Adama, AddisAbaba) and Southern (Hawassa and Wolayta) Ethiopia according to the procedures kept in Quinn *et al* (2002) and a total of 205 species were isolated and evaluated using disk diffusion method for their antimicrobial susceptibility to 10 different antimicrobial drugs which were Amoxicillin, Ciprofloxacin, Tetracycline, Erythromycin, Nalidixic Acid, Nitrofurantoin, Streptomycin, Penicillin G, Sulfamethoxazole- Trimethoprim and Vancomycin. Antimicrobial susceptibility testing was carried out in accordance with the guidelines published by the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards, 2014).

III. Result

In this study, *Staphylococcus* were found to be highly susceptible to Ciprofloxacin (85.4%) followed by Sulfamethoxazole-Trimethoprim (68.8%). However these isolates were highly resistant to Penicillin G (94.1%) and Tetracycline (79%) followed by Amoxicillin (60.5%). The antimicrobial resistance profiles of *Staphylococcus* at genus level and by species level are shown in Table 1 and Table 2, respectively.

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Table 1 : Resistance of *Staphylococcus* isolates to different antimicrobials (n = 205)

Antimicrobials	Resistant	Intermediate	Susceptible
	No. (%)	No. (%)	No. (%)
Amoxicillin	124(60.5)	-	81(39.5)
Ciprofloxacin	9(4.4)	21(10.2)	175(85.4)
Tetracycline	162(79)	12(5.9)	31(15.1)
Erythromycin	115(56.1)	57(27.8)	33(16.1)
Nalidixic Acid	80(39)	34(16.6)	91(44.4)
Nitrofurantoin	118(57.6)	39(19)	48(23.4)
Streptomycin	116(56.6)	36(17.6)	53(25.9)
Penicillin G	193(94.1)	-	12(5.9)
Sulfamethoxazole-Trimethoprim	42(20.5)	22(10.7)	141(68.8)
Vancomycin	122(59.5)	-	83(40.5)

From all *Staphylococci* isolates tested for drug susceptibility pattern, only 1 isolate (*S. aureus*) was susceptible to all tested drugs. Seven isolates were resistant to only one drug whereas 4 (3 *S. aureus* and 1 *S. hycus*) were resistant to Penicillin G and three *S. aureus* isolates were resistant to Tetracycline, Erythromycin and Nalidixic acid (each isolate for single drug). Coagulase negative *Staphylococci* were highly resistant to all tested drugs except Ciprofloxacin (0%). S. *aureus* were also highly resistant to Penicillin (92.2%), Tetracycline (74.5%), Amoxicillin (58.8%), Vancomycin (56.9%), Erythromycin (55.6%), Streptomycin (53.6%) and Nitrofurantoin (52.3%) (Table 2).

Table 2 : Resistance of *Staphylococcus* Species to different antimicrobials

Antimicrobials	Staph species	No of species	Resistance	Intermediate	Susceptible
			No (%)	No (%)	No (%)
Amoxicillin	CNS	35	25(71.4%)	-	10(28.6)
	S. *aureus*	153	90(58.8)	-	63(41.2)
	S. *hycus*	11	5(45.5)	-	6(54.5)
	S. *intermedius*	6	4(66.7)	-	2(33.3)
Ciprofloxacin	CNS	35	0(0%)	0(0%)	35(100%)
	S. *aureus*	153	7(4.6%)	20(13.1%)	126(82.4%)
	S. *hycus*	11	1(9.1%)	0(0%)	10(90.9%)
	S. *intermedius*	6	1(16.7%)	1(16.7%)	4(66.7%)
Tetracycline	CNS	35	33(94.3%)	1(2.9%)	1(2.9)
	S. *aureus*	153	114(74.5)	11(7.2%)	28(18.3)
	S. *hycus*	11	9(81.8)	0(0%)	2(18.2)
	S. *intermedius*	6	6(100%)	0(0%)	0(0%)
Erythromycine	CNS	35	21(60.0%)	12(34.3)	2(5.7%)
	S. *aureus*	153	85(55.6%)	42(27.5%)	26(17%)
	S. *hycus*	11	7(63.6%)	0(0%)	4(36.4%)
	S. *intermedius*	6	2(33.3%)	3(50%)	1(16.7%)
Nalidixic acid	CNS	35	21(60%)	7(20%)	7(20%)
	S. *aureus*	153	53(34.6%)	25(16.3)	75(49%)
	S. *hycus*	11	3(27.3%)	1(9.1%)	7(63.6%)
	S. *intermedius*	6	3(50%)	1(16.6)	2(33.3%)
Nitrofurantoin	CNS	35	30(85.7%)	1(2.9%)	4(11.4%)
	S. *aureus*	153	80(52.3%)	33(21.6%)	40(26.1%)
	S. *hycus*	11	4(36.4)	5(45.5%)	2(18.2%)
	S. *intermedius*	6	4(66.7)	0(0%)	2(33.3%)
Streptomycin	CNS	35	26(74.3%)	4(11.4%)	5(14.3%)
	S. *aureus*	153	82(53.6%)	29(19%)	42(27.5%)
	S. *hycus*	11	2(18.2%)	3(27.3%)	6(54.5%)
	S. *intermedius*	6	6(100%)	0(0%)	0(0%)
Penicillin G	CNS	35	35(100%)	-	0(0%)
	S. *aureus*	153	141(92.2%)	-	12(7.8%)
	S. *hycus*	11	11(100%)	-	0(0%)
	S. *intermedius*	6	6(100%)	-	0(0%)

Sulfamethoxazole - Trimethoprim	CNS	35	12(34.3%)	5(14.3%)	18(51.4%)
	S. *aureus*	153	26(17%)	17(11.1)	110(71.9%)
	S. *hycus*	11	0(0%)	0(0%)	11(100%)
	S. *intermedius*	6	4(66.7)	0(0%)	2(33.3%)
Vancomycin	CNS	35	28(80%)	-	7(20%)
	S. *aureus*	153	87(56.9%)	-	66(43.1%)
	S. *hycus*	11	4(36.4%)	-	7(63.6%)
	S. *intermedius*	6	3(50%)	-	3(50%)

a) *Double Antimicrobial Resistance of the Isolated Staphylococcus*

193 isolates were resistant to Penicillin G and 162 isolates were resistant to Tetracycline. The resistant isolates for two drugs Penicillin G and Tetracycline were 49. Therefore 113 isolates were resistant to Tetracycline and 144 isolates were resistant to Penicillin G without sharing each other (Table 3).

Table 3 : *Staphylococcus* isolates (n= 205) drug resistance pattern as assessed for single (shaded diagonal), double drug resistance (below diagonal) and the unshared isolate number in the double resistance (above diagonal)

	AML	CIP	TE	E	NA	F	S	P	SXT	VA
AML	124	117(2)	28(66)	56(47)	78(34)	56(50)	48(40)	0(69)	100(18)	49(47)
CIP	7	9	1(154)	2(108)	3(74)	3(112)	4(111)	0(184)	7(40)	7(120)
TE	96	8	162	64(17)	86(4)	54(10)	59(13)	113(144)	124(4)	56(16)
E	68	7	98	115	77(42)	42(45)	50(51)	7(85)	82(9)	40(47)
NA	46	6	76	38	80	21(59)	21(57)	4(117)	58(20)	22(64)
F	68	6	108	73	59	118	33(31)	2(77)	82(6)	32(36)
S	76	5	103	65	59	85	116	5(82)	82(8)	38(44)
P	124	9	49	108	76	116	111	193	154(3)	74(3)
SXT	24	2	38	33	22	36	34	39	42	9(89)
VA	75	2	106	75	58	86	78	119	33	122

AML: Amoxicillin, CIP: Ciprofloxacin, TE: Tetracycline, E: Erythromycin, NA: Nalidixic Acid, F: Nitrofurantoin, S: Streptomycin, P: Penicillin, SXT: Sulfamethoxazole - Trimethoprim, VA: Vancomycin

b) *Multidrug Resistance Pattern of Staphylococci Species*

Out of 153 *S. aureus* isolates screened against 10 different drugs, 146 isolates had resistance to $\geq$ 2 drugs. However, 6 isolates had single drug resistance whilst 1 isolate was susceptible to all drugs. Of the 146 *S. aureus* isolates that had resistance to $\geq$2 drugs, 11 isolates were resistant to 2 drugs, 17 isolates to 3 drugs, 25 isolates to 4 drugs, 31 isolates to 5 drugs, 21 isolates to 6 drugs, 37 isolates to 7 drugs and 4 isolates were resistant to 9 drugs (Table 4).

Table 4 : Multidrug resistance pattern of S. *aureus*

No of drug	Pattern (isolate)	No. of drug	Pattern (isolate)	No. of drug	Pattern (isolate)
2	AMLP (4)		AMLFSP(1)		TEENAFSP(1)
	FP(1)		AMLNAPVA(1)		TEEFPSXTVA(1)
	TENA(1)		AMLTEPVA(1)		TEENAFPVA(2)
	TEP(4)		ESPVA(1)		AMLTEEFPVA(3)
	SP(1)		TESPVA(1)		AMLTEEFSP(2)
3	TENAP(1)		AMLTEFP(1)		TEESPSXTVA(1)
	TENAVA(1)		TEESP(1)		AMLTEESPSXT(1)
	FPVA(1)	5	TENASPVA(1)		AMLTESPSXTVA(1)
	TEENA(1)		TENAFPVA(1)		AMLTEESPVA(2)
	AMLSP(4)		TEEFSP(5)	7	AMLTENAFSPVA(10)
	TEEF(1)		AMLEFPSXT(1)		TEEFSPSXTVA(4)
	TEEP(1)		AMLTEENAP(1)		AMLTEEFSPVA(3)
	ESP(1)		TEEFSSXT(1)		AMLCIPTEENAFP(1)
	TEPVA(2)		TEEFPVA(4)		AMLTEENASPVA(2)
	EFP(1)		AMLTEESP(1)		CIPTEENAFSP(2)
	EPVA(2)		AMLTENASP(1)		TEENAFSPSXT(2)
	AMLTEP(1)		AMLTEFSP(3)		AMLCIPTEENAFP(1)
4	FPSXTVA(1)		AMLTEEFP(3)		AMLTEENAPSXTVA(1)
	AMLSPVA(1)		AMLTEEPVA(4)		AMLCIPTEENAPVA(1)
	AMLTEES(1)		AMLTEPSXTVA(1)	8	AMLTEENAFSPVA(5)
	AMLEPVA(7)		AMLTESPVA(2)		AMLENAFSPSXTVA(1)
	AMLTESP(1)		TEFSPVA(1)		AMLTENAFSPSXTVA(1)
	AMLTEEP(1)		TEESPVA(1)		AMLCIPTEEFSPSXT(1)
	TEFPVA(2)	6	TENAFSPVA(4)		TEENAFSPSXTVA(2)
	AMLFSP(2)		TENAFSPVA(1)	9	AMLTEENAFSPSXTVA(3)
	AMLTENAP(2)		TEEFSSXTVA(2)		AMLCIPTEEFSPSXTVA(1)

AML: Amoxicillin, CIP: Ciprofloxacin, TE: Tetracycline, E: Erythromycin, NA: Nalidixic Acid, F: Nitrofurantoin, S: Streptomycin, P: Penicillin, SXT: Sulfamethoxazole - Trimethoprim, VA: Vancomycin

From 35 CNS isolates tested for drug resistance pattern 1 isolate was resistant to 3 drugs, 3 isolates to 4 drugs, 6 isolates to 5 drugs, 6 isolates to 6 drugs, 8 isolates to 7 drugs, 5 isolates to 8 drugs and 6 isolates were resistant to 9 drugs. From a total of 11 *S. hycus* isolates that were subjected to drug susceptibility test, 1 isolate was resistant to single drug and 10 isolates were multidrug resistant. From those isolates 2 isolates were resistant to 3 drugs, 4 isolates to 4 drugs, 2 isolates to 5 drugs, 1 isolate to 6 and 7 drugs each. Out of a total of 6 *S.intermedius* isolates that were tested for their drug resistancepattern, 1 isolate were resistant to 5 drugs, 2 isolates to 6 drugs, 2 isolates to 7 drugs and 1 isolate was resistant to 8 drugs (Table 5).

Table 5 : Multidrug resistance pattern of CNS, S. *intermidius* and S. *hycus*

No of drug	Pattern (isolates) CNS	No of drug	Pattern (isolates) *S.intermedius*
3	TENAP(1)	5	AMLCIPTESP (1)
4	FSPVA(1)	6	TENAFSPVA(1)
	AMLTEFP(1)		AMLTEESPSXT(1)
	AMLESP(1)	7	TENAFSPSXTVA(1)
5	AMLTENASP(1)		AMLTENAFSPSXT(1)
	TENAFPVA(2)	8	AMLTEEFSPSXTVA(1)
	AMLTEEFP(1)		
	AMLTEEPVA(1)	No of drug	Pattern (isolates) *S.hycus*
	TEENAPVA(1)	1	P(1)
6	TENAFSPVA(2)	3	AMLTEP(1)
	AMLTEFSPVA(2)		TEEP(1)
	AMLTEEFPVA(1)	4	AMLTEEP(2)
	AMLTEENAFP(1)		AMLCIPNAP(1)
7	AMLTENAFSPVA(3)		TEENAP(1)
	TENAFSPSXTVA(1)	5	TEEFPVA(2)
	AMLTEEFSPVA(3)	6	TEEFSPVA(1)
	AMLTEEFSPSXT(1)	7	AMLTENAFSPVA(1)
8	AMLTEEFSPSXTVA(2)		
	TEENAFSPSXTVA(2)		
	AMLTEENAFSPVA(1)		
9	AMLTEENAFSPSXTVA(6)		

AML: Amoxicillin, CIP: Ciprofloxacin, TE: Tetracycline, E: Erythromycin, NA: Nalidixic Acid, F: Nitrofurantoin, S: Streptomycin, P: Penicillin, SXT: Sulfamethoxazole - Trimethoprim, VA: Vancomycin

IV. Discussion

In the present study *Staphylococcus* were found to be highly susceptible to Ciprofloxacin (85.4%) followed by Sulfamethoxazole-Trimethoprim (68.8%). However these isolates were highly resistant to Penicillin G (94.1%) and Tetracycline (79%) followed by Amoxicillin (60.5%). This result indicated that most of the *Staphylococci* isolates were susceptible to Ciprofloxacin which is lower than the result of Suleiman *et al*. (2013) who reported that 100% isolates were susceptible to Ciprofloxacin.

Most researches were directed to antibiotic resistance of *Staphylococci* isolated from food producing animals and their products focusing on the *S. aureus* species, whereas less attention is paid to the group of coagulase-negative *Staphylococci*. In this study coagulase negative *Staphylococci* were highly resistant to all tested drugs except Ciprofloxacin (0%). The result of Heba *et al*. (2014) reported that 33.3 % of CNS was resistant to Ciprofloxacin. This could be due to the differences in source of the isolated CNS in the different study areas. The present study presented that CNS were resistant to Penicillin G (100%), Tetracycline (94.3%), Nitrofurantoin (85.7%), Vancomycin (80%), Streptomycin (74.3%), Amoxicillin (71.4%), Erythromycin (60%), Nalidixic acid (60%) and Sulfamethoxazole – Trimethoprim (34.3%). Results of a study by Heba *et al*., (2014) showed that 87% of coagulase negative *Staphylococci* strains were resistant to Erythromycin that is higher than the present study (60%).

S.aureus were also highly resistant to Penicillin G (92.2%), Tetracycline (74.5%), Amoxicillin (58.8%), Vancomycin (56.9%), Erythromycin (55.6%), Streptomycin (53.6%) and Nitrofurantoin (52.3%). This finding is in accordance with Abera *et al.* (2013) who reported *S.aureus* isolates resistant to Penicillin G were 94.4%. The present study disagrees with the result of Koksal *et al*. (2007) who found 0% resistance of *S.aureus* to Vancomycin. The differences in those results might be due to the differences in sample source and sample type of the isolates that were subjected to the test.

From all *Staphylococci* isolates tested for drug susceptibility pattern, only 1 isolate (*S.aureus*) was

susceptible to all tested drugs and 99.51% were resistant to at least one of the antibiotics tested. This finding is higher than the result of Geidam *et al*. (2012) who reported the result of 33.4% of *Staphylococci* were resistant to at least one of the antibiotics tested. This difference could be due to the differences in type of species isolated and type of drugs used on susceptibility test or due to the differences on the intensity of drug use and misuse. Out of 153 *S. aureus* isolates screened against 10 different drugs, 107 isolates (69.93%) were resistant to $\geq$ 4 drugs. This finding is comparable with the report of Geidam *et al*. (2012) who reported a total of 77.2% of *S.aureus* isolates that were resistant to $\geq$ 4 drugs.

V. Conclusion

According to the present study the *Staphylococci* species isolated from poultry were resistant to almost all drugs in which all *Staphylococci* were multidrug resistant except one isolate. The indiscriminate use of antimicrobial agents for prophylactic as well as other therapeutic purpose could be the reasons for increased antimicrobial resistance of *Staphylococci*. Exchange of resistance encoding genes among *Staphylococci* from different reservoirs (humans, poultry, and poultry products) is possible, but it is not known to what level this happens. Not only that chickens are at risks, poultry farm and abattoir workers and consumers are equally exposed to serious hazards due to multidrug resistance *Staphylococci*. Therefore restrictions on the irrational use of antibiotics should be applied and establishment of standardized monitoring systems in poultry farms are required. The extent of exchange of resistance encoding genes among *Staphylococci* from humans, poultry and poultry products in Ethiopia has to be investigated extensively.

VI. Acknowlegements

The authors would like to thank Southern Agricultural Research Institute, Addis Ababa University and Wolayta Sodo Regional Veterinary Laboratory for financial support and laboratory work.

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