AMR PROFILING OF SALMONELLA IN HUMANS AND CHICKEN IN SENEGAL

Dr Cheikh Fall
Institut Pasteur de Dakar

Strenghtening the One Health approach to AMR Surveillance

Friday, 1st July 2022

BACKGROUND

Salmonella: major common foodborne pathogen isolated from food-producing animals

>2600 serovars identified so far

Salmonella is transmitted to humans along the farm-to-fork continuum

Some Salmonella serovars are "host-restricted" whereas others have broad host spectrum known as "host-adapted" serovars.

Clinically, Salmonella infections are characterized:

Gastro-enteritis: not fatal and are caused by several serovars

Invasive diseases: Typhoid fever (serovars Typhi and paratyphi); invasive non-Typhoidal Salmonella (iNTS)

BACKGROUND

Typhoid fever and iNTS are estimated to cause over 25 million cases and nearly 900,000 deaths annually (Marks F et al, 2017)

Data on Typhoid fever and iNTS are scarce in Africa, only sporadic cases have been reported

Available data indicate that iNTS is a significant cause of invasive disease in sSA, with Typhimurium and Enteritidis being the most frequently isolated serovars (Gilchrist JJ et al, 2019; MacLennan CA et al, 2017)

The challenge is the reduction of Salmonellosis treatment options and the increasing AMR clone.

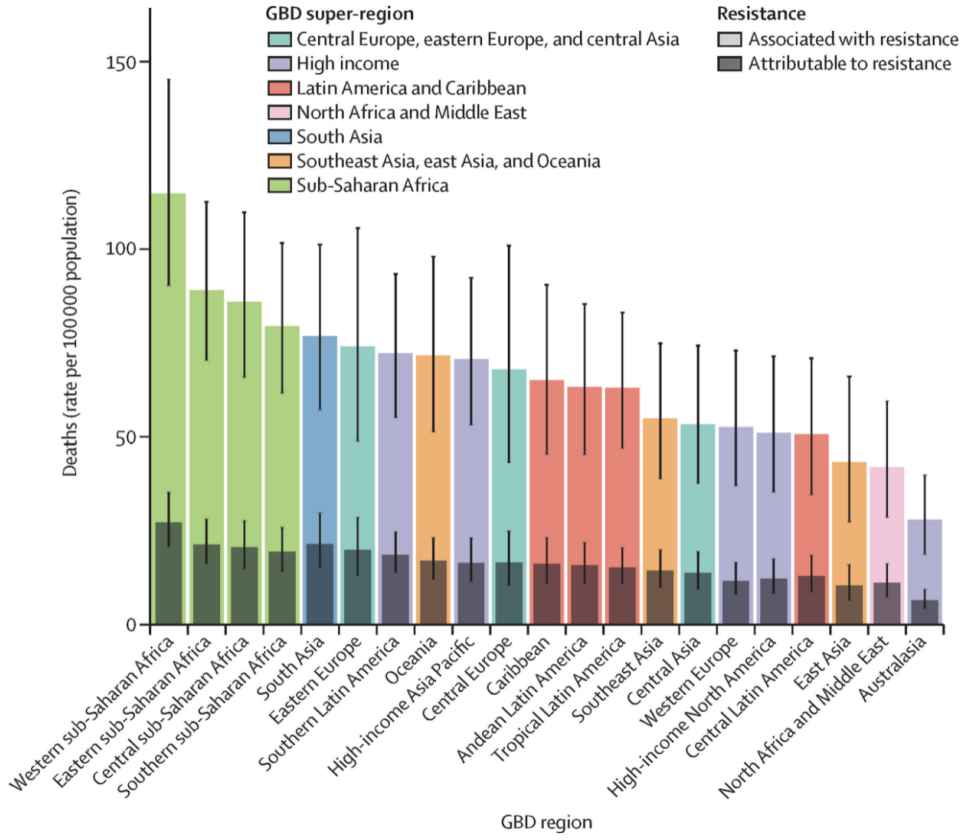
Food production, especially in poultry represent potential risks in emerging and disseminating drug-resistant Salmonella, with multi-drug resistant (MDR) strains.

BACKGROUND

AMR is a global crisis:

The Lancet systematic review estimated the global burdun of AMR far greather than the WHO predictions of 700,000 deaths per year and 10 million deaths by 2050 (and its economic impact) as far more believable scenario (O'Neil report, 2014)

It was estimated to 4.95 million deaths in 2019 (Murray et al, 2022)



OBJECTIVE

The aim of the study was to determine the resistance profile of non-typhoidal Salmonella strains from clinical salmonellosis cases in humans, and the potential transmission of these strains from retail chicken meat.

We were interested to investigate serotypes distribution, phylogenic relationship, phenotypic and genotypic AMR profiles, and virulence and plasmid composition of isolates from the two sources analyzed.

METHODOLOGY

Sampling was done in Dakar, through a survey conducted between July 2012-June 2013.

Salmonella strains were collected from chicken carcasses sold at multipurposed markets and from diarrheic stools from patients visiting IPD

Culture was done using RVE medium for enrichment and isolation with XLD and Hecktoen media.

Isolate strains were confirmed using API 20 E and AST realized based on CASFM according to manufacturer recommendations

Serotyping was performed by slide agglutination in a Kauffmann–White scheme (Grimont and Weill, 2007)

METHODOLOGY

A set of **72 Salmonella isolates** recovered from humans (n = 19) and chicken meat (n = 53), including different serotypes were selected for genomic study

Genomic DNA extraction using the MasterPure DNA kit and DNA libraries prepared with the Nextera XT DNA libraries kit

Sequencing was performed using Illumina platform (MiSeq)

QC on generated data, followed *de novo* assembling with Shovill v1.1.0 using **SPAdes v3.14.1**

Plasmids detection was done with **plasmidSPAdes** genome assembler v3.14.1

Resistance and virulence genes were investigated using online platforms (CARD, ResFinder, Abricarde, VFDB, ….)

Serotyping was determined with **SeqSero 2** and Sequence type with **MLST database**

Phylogeny was done based on SNPs from the core genome with **CSI Phylogeny 1.4** (Center for Genomic Epidemiology) and visualized with **R Studio software**

RESULTS

Serotypes distribution:

24 serotypes identified, mainly Brancaster (14), Kentucky (13), Hadar (11), Chester (4), Schwarzengrund (4), and Senftenberg (4);

Isolates from human were diverse (16 serotypes vs 10)

Some serotypes like Banana and Gaminara were found in >1 people: possible silent outbreak

Serotypes	Human	Chicken	Total
Brancaster	0	14	14
Kentucky	1	12	13
Hadar	0	11	11
Chester	0	4	4
Schwarzengrund	1	3	4
Senftenberg	0	4	4
Banana	3	0	3
Gaminara	2	0	2
Johannesburg	0	2	2
Isangi	1	0	1
Give	1	0	1
Poona	1	0	1
Corvallis	1	0	1
Somone	1	0	1
Muenster	1	0	1
Baildon	1	0	1
Oranienburg	1	0	1
3,10:e,h:-	1	0	1
Virchow	1	0	1
Rissen	1	0	1
Okerara	1	0	1
Typhimurium	0	1	1
Brandenburg	0	1	1
Vejle	0	1	1
Total	19	53	72

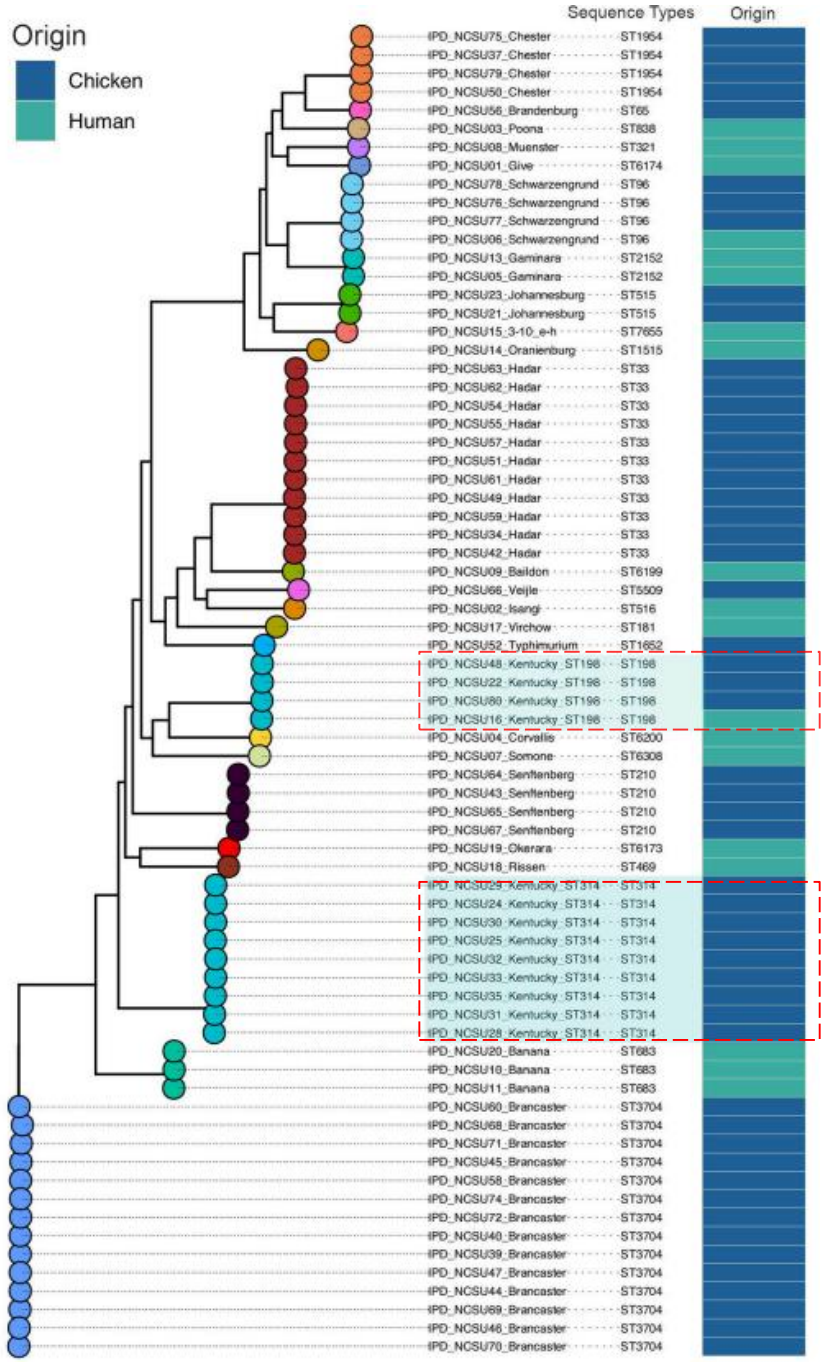
RESULTS

Phylogenic diversity and source of Salmonella isolates:

Samples were clustered according to serotypes, except for Kentucky belonging to STs 198 and 314

Kentucky ST198 is an emerging clone, harboring genomic island with MDR determinants

Kentucky ST198 and Schwarzengrund ST96 were found both from human and chicken sources



RESULTS

Antimicrobial susceptibility testing:

Resistance to historically 1st and 2nd line Salmonella treatment was rare, except to SXT (38,9%),

In contrast, it was high to quinolone (19,4%), macrolide (30,6%) and cyclines (48,6%).

MDR was found on 27,8% (20/72) isolates, including 1 respecting MDR Salmonella definition (Serotype Poona).

Resistance was more frequent from chicken isolates (83% vs 10,5%).

This raises the selection pressure exerted by the use of antimicrobials as a growth promoter in chicken farming

Classes	Antimicrobials	Origin and number of resistant isolates		Total
		Human	Chicken	
Phenicol	Chloramphenicol	1	0	1
Anti-folate	Sulfamethoxazol +Trimethoprim	1	27	28
Penicillin	Ampicillin	2	1	3
	Ticarcillin	2	1	3
	Amoxicillin + Clavulanic Acid	1	0	1
Monobactam	Aztreonam	1	0	1
Cephem	Cefalothin	1	0	1
	Cefoxitin	1	0	1
	Cefotaxime	1	0	1
	Ceftazidime	1	0	1
	Cefepime	0	0	0
Carbapenem	Imipenem	0	0	0
Aminoglycoside	Gentamicin	2	3	5
	Kanamycin	0	2	2
	Tobramycin	1	0	1
Quinolone	Nalidixic Acid	1	14	15
	Ciprofloxacin	1	14	15
	Norfloxacin	1	2	3
	Ofloxacin	1	12	13
Cycline	Tetracycline	1	35	36
Polymixin	Colistin	0	0	0
Macrolide	Erythromycin	2	22	24

RESULTS

AMR profile and resistance genes:

22 ARG and 4 mutations associated to resistance to **quinolone**, including 2 *parC* and 2 *gyrA* genes were found

Genes encoding **aminoglycoside** modifying enzymes were the most frequently found ARG with 10 genes detected in 34 isolates;

acetyltransferase (*aac(6')-Iaa*) detected in all the isolates without conferring resistance: cryptic gene present in most Salmonella isolates;

Phosphotransferase subfamily (*aph(3')-Ia, aph(3')-VIa, aph(3")-Ib, aph(6)-Id*), not associated to AMR

Serotypes	AMR Phenotypic Profile	ARG[&]	Plasmidic ARG
Brancaster	(Kn) Na Cp Ox Te Er	*sul2 tetB aph(3')-Ib aph(3")-Ib aph(6)-Id qnrB19*	*sul2 tetB qnrB19 tetD*
Brancaster	(Kn) Na Cp Ox Te Er	*sul2 tetB aph(3')-Ib aph(3")-Ib aph(6)-Id qnrB6*	*qnrB19*
Brancaster (X 4)[#]	ST Te	*sul2 dfrA1 tetB aph(3')-Ib aph(3")-Ib aph(6)-Id*	
Brancaster (X 4)[#]	ST Te	*sul2 dfrA1 tetB aph(3')-Ib aph(3")-Ib aph(6)-Id*	*sul2 dfrA1 aph(3")-Ib aph(6)-Id*
Brancaster	Kn (Tm) Te Er	*sul2 tetB aph(3')-Ib aph(3")-Ib aph(6)-Id*	
Brancaster	ST (Kn) Na Cp Te Er	*sul2 dfrA1 dfrA15 tetA tetB aph(3')-Ib aph(3")-Ib aph(6)-Id qnrB19*	*sul2 dfrA1 tetA aph(3")-Ib aph(6)-Id qnrB19*
Brancaster	Te	*sul2 tetB aph(3')-Ib aph(3")-Ib aph(6)-Id*	
Brancaster	(Kn) Te Er	*sul2 tetB aph(3')-Ib aph(3")-Ib aph(6)-Id*	
Kentucky ST198	Ap Tc Gm (Tm) Na Cp No Ox Te Er	*aac(3)-Id aadA7 tetA sul1 blaTEM-1b parC-S80I gyrA-S83F, gyrA-D87N*	
Kentucky ST198	ST Na Cp No Ox Te Er	*sul2 dfrA14 tetA aph(6)-Id parC-S80I*	*sul2 dfrA14 tetA aph(6)-Id*
Kentucky ST198	ST Ap Tc AC Gm Na Cp No Ox Te Er	*aac(3)-Id aadA7 blaTEM-1B sul1 tetA parC-S80I gyrA-S83F, gyrA-D87N*	
Kentucky ST198	ST Na Cp Ox Te Er	*sul2 dfrA14 tetA aph(6)-Id parC-S80I gyrA-S83F, gyrA-D87N*	*sul2 dfrA14 tetA aph(6)-Id*
Kentucky ST314 (X 2)[#]	Er	*sul1 dfrA15**	
Kentucky ST314	ST Gm Te Er	*aac(3)-Id aadA7 sul1 dfrA15 tetA aph(3")-Ib aph(6)-Id*	
Kentucky ST314 (X 6)[#]	ST	*sul1 dfrA15*	
Hadar (X 2)[#]	ST Na Cp (Ox) Te Er	*sul2 dfrA1 tetA aph(3")-Ib aph(6)-Id qnrB19*	*sul2 dfrA1 aph(3")-Ib aph(6)-Id qnrB19*
Hadar	Te Er	*sul2 dfrA1** *tetA aph(3")-Ib aph(6)-Id*	
Hadar (X 6)[#]	Te	*tetA aph(3")-Ib aph(6)-Id*	
Hadar	ST Gm (Tm) Na Cp Ox Te Er	*sul2 dfrA1 tetA aph(3")-Ib aph(6)-Id qnrB19*	
Hadar	Te	*tetA aph(3")-Ib*	

ARG, antimicrobial resistance gene. Antimicrobials : AC, amoxicillin + clavulanic acid; Ap, ampicillin; At, aztreonam; Cf, cefalothin; Cx, cefoxitin; Ct, cefotaxime; Cz, ceftazidime; Cm, chloramphenicol; Cp, ciprofloxacin; Er, erythromycin; Fp, cefepime; Co, colistin; Gm, gentamicin; Ip, imipenem; Kn, kanamycin; Na, nalidixic acid; No, norfloxacin; Ox, ofloxacin; ST, sulfamethoxazole + trimethoprim; Tc, ticarcillin; Te, tetracycline; Tm, tobramycin.

[&], gene *aac(6')-Iaa*, not shown in the Table, was present in all isolates without conferring AMR

*, *sul* and *dfrA* genes present in isolates susceptible to sulfamethoxazole + trimethoprim.

[#], Number of isolates that have the same resistance phenotype and ARG profile.

Isolates from humans are shaded

RESULTS

AMR profile and resistance genes (end):

28/72 isolates displaying resistance to **SXT** harbored *sul1, sul2* or *dfrA* gene

The Poona serotype resistant to **chloramphenicol** possessed a *floR* ARG

Resistant isolates to **tetracycline** harbored *tetA* or *tetB* efflux pump (36/72)

Resistance to **ESBL** was supported by

blaOXA-10, blaTEM-1, blaDHA-1 and *blaCMY-2*

fosA gene were found in 7 isolates, even as **fosfomycine** drug was not tested.

Serotypes	AMR Phenotypic Profile	ARG&	Plasmidic ARG
Chester	ST (Kn) Na Cp Ox Te Er	*sul2 dfrA14 tetA aph(6)-Id qnrB19*	
Chester	sensitive		
Chester	ST Na Cp Ox Te Er	*sul2 dfrA14 tetA aph(6)-Id qnrB19*	*sul2 dfrA1 tetA aph(6)-Id*
Chester	ST Na Cp Ox Te Er	*sul2 dfrA14 tetA aph(6)-Id qnrB19*	
Schwarzengrund	sensitive		
Schwarzengrund (X 2)#	sensitive		
Schwarzengrund	Er		
Senftenberg (X 4)#	sensitive		
Banana (X 3)#	sensitive	*fosA*	
Gaminara (X 2)#	sensitive		
Johannesburg	ST Na Cp Ox Te Er	*sul1 dfrA7 tetA gyrA-S83F*	
Johannesburg	ST Kn Na Cp Ox Te Er	*sul1 dfrA7 tetA gyrA-S83F*	
Isangi	sensitive		
Give	sensitive		
Poona	Cm ST Ap Tc AC At Cf Cx Cz Ct Gm Tm Er	*aadA1 aadA2b ant(2")-Ia aph(3')-Ia aph(3')-Via arr-2 sul1 sul2 floR blaDHA-1 blaOXA-10 dfrA14*	*aph(3')-Ia aph(3')-VIa sul1 sul2 floR blaDHA-1*
Corvallis	sensitive		
Somone	sensitive	*fosA*	
Muenster	sensitive		
Baildon	sensitive		
Oranienburg	sensitive	*fosA*	
3,10:e,h:-	sensitive	*fosA*	
Virchow	sensitive		
Rissen	sensitive		
Okerara	sensitive		
Typhimurium	Ox Te Er	*fosA tetA qnrB7*	*tetA qnrB7*
Brandenburg	sensitive		
Vejle	sensitive		

ARG, antimicrobial resistance gene. Antimicrobials : AC, amoxicillin + clavulanic acid; Ap, ampicillin; At, aztreonam; Cf, cefalothin; Cx, cefoxitin; Ct, cefotaxime; Cz, ceftazidime; Cm, chloramphenicol; Cp, ciprofloxacin; Er, erythromycin; Fp, cefepime; Co, colistin; Gm, gentamicin; Ip, imipenem; Kn, kanamycin; Na, nalidixic acid; No, norfloxacin; Ox, ofloxacin; ST, sulfamethoxazole + trimethoprim; Tc, ticarcillin; Te, tetracycline; Tm, tobramycin.
&, gene *aac(6')-Iaa*, not shown in the Table, was present in all isolates without conferring AMR
*, *sul* and *dfrA* genes present in isolates susceptible to sulfamethoxazole + trimethoprim.
#, Number of isolates that have the same resistance phenotype and ARG profile.
Isolates from humans are shaded

RESULTS

Plasmid presence and content in Salmonella isolates:

Plasmid was found in **55,6%** isolates (42.1% Human and 60.4% Chicken), mostly among Brancaster (64.3%), Hadar (81.8%), Senftenberg (100%), and Schwarzengrund (75%) serotypes.

Kentucky ST198 harbored plasmid, in contrast to ST314

Plasmids harboring ARG were found on 15 genomes

Plasmids harbored virulence genes of pathogenic E. coli were also found:

IncI1, harbored *pic* found on EAEC and Shigella

IncFII, harbored *faeEDC* complex found ETEC

Plasmids harbored *pap* genes of UPEC was also found

Serotypes	Plasmid replicons	ARG	Virulence genes
Brancaster	Col440I	sul2 tetB qnrB19 tetD	papI, papB
Brancaster	Col440I	qnrB19	
Brancaster	ColRNAI	sul2 dfrA1 aph(3")-Ib aph(6)-Id	
Brancaster	ColRNAI / Col440I Col156 ColpVC	sul2 dfrA1 tetA aph(3")-Ib aph(6)-Id qnrB19	
Brancaster	ColRNAI	sul2 dfrA1 aph(3")-Ib aph(6)-Id	
Brancaster	ColRNAI	sul2 dfrA1 aph(3")-Ib aph(6)-Id	
Brancaster	ColRNAI	sul2 dfrA1 aph(3")-Ib aph(6)-Id	
Kentucky ST198	ColRNAI / Col440II	sul2 dfrA14 tetA aph(6)-Id	
Kentucky ST198	ColRNAI / Col440II	sul2 dfrA14 tetA aph(6)-Id	
Hadar	ColRNAI / Col440II Col156	sul2 dfrA1 aph(3")-Ib aph(6)-Id qnrB19	
Hadar	ColRNAI / Col440I	sul2 dfrA1 aph(3")-Ib aph(6)-Id qnrB19	
Chester	ColRNAI / Col440I Col156	sul2 dfrA1 tetA aph(6)-Id	
Schwarzengrund	IncI2 IncFIB IncP1	sul2 tetA aph(3')-Ia aph(3")-Ib aph(6)-Id	papI, papB, papH, papDJKEFG
Typhimurium	IncX3 / IncY ColRNAI / Col440	tetA qnrB7	
Poona	IncA/C	aph(3')-Ia aph(3')-VIa sul1 sul2 floR blaDHA-1	
Gaminara	IncI1	ND	pic, astA
Gaminara	IncFII	ND	faeEDC
Johannesburg	ColRNAI / Col440II ColpVC	ND	papDJKEFGC

ARG, antimicrobial resistance gene; pap, pyelonephritis-associated pili; (pic) serine protease precursor [Pic (VF0232)] [Escherichia coli CFT073]; (astA) heat-stable enterotoxin 1 [EAST1 (VF0216)] [Escherichia coli O44:H18 042]; fae, gene encoding a component of the K88 fimbriae.
ND, no ARG detected. Isolates from Human are shaded

DISCUSSION

WGS approach is better suited to compare isolates from different sources and appreciated for animal human transmission

Salmonella contamination of chicken and their derivation are frequent in Senegal, as previously reported (Cardinale E. et al, 2004; Missohou A. et al, 2011; Pouillot R. et al, 2012), even as transmission to human was rarely mentioned. Besides chicken, Salmonella has been detected in several food production in Senegal and in wildlife species.

High diversity of serovars among Human isolates, highlighted their ability to cause clinical infections

Endemicity of Salmonella and serotypes diversity are a major public health concern, especially in the context of the rising frequency of iNTS in sSA

DISCUSSION

WGS data in this study were consistent with phenotype AMR, which were predominant among chicken isolates and rises with the high frequency of AMU in farming for production.

Resistance to 1st line antimicrobials used to treat clinical salmonellosis, including SXT and fluoroquinolones was high and was concordant to previous results (Crump JA et al, 2015).

Resistance to β-lactam (replaced quinolone for treatment) was low. This finding is consistent with available data in Senegal.

A robust and integrated surveillance system is needed to tackle AMR and implement appropriate countermeasures;

WGS methodology can be a good approach and can be based on a one health approach to identify sources of contamination and of AMR dissemination. This will contribute to keep active molecules available for treatment of bacterial infections

PUBLICATIONS

PLOS ONE

RESEARCH ARTICLE

Genomics of human and chicken *Salmonella* isolates in Senegal: Broilers as a source of antimicrobial resistance and potentially invasive nontyphoidal salmonellosis infections

Yakhya Dieye[1,2]*, Dawn M. Hull[3], Abdoul Aziz Wane[1], Lyndy Harden[3], Cheikh Fall[1], Bissoume Sambe-Ba[1], Abdoulaye Seck[1], Paula J. Fedorka-Cray[3], Siddhartha Thakur[3]

1 Pole of Microbiology, Institut Pasteur, Dakar, Sénégal, **2** Département Génie Chimique et Biologie Appliquée, École Supérieure Polytechnique, Université Cheikh Anta Diop, Dakar, Sénégal, **3** Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, United States of America

Check for updates

Foodborne Pathogens and Disease, Vol. 16, No. 2 | Original Articles

Antimicrobial Resistance Profile of *Salmonella* Isolates in Chicken Carcasses in Dakar, Senegal

Ndeye Khota Fall-Niang, Bissoume Sambe-Ba, Abdoulaye Seck, Saidou Nourou Deme, Abdoul Aziz Wane, Raymond Bercion, Rianatou Alambedji-Bada, and Amy Gassama-Sow

Published Online: 13 Feb 2019 | https://doi.org/10.1089/fpd.2018.2459

Thank you for your attention!

Your questions, please