

# Resistotyping of *Salmonella* spp. and *Staphylococcus aureus* from milk and milk products sold in Sabon-gari and Zaria Local Government Areas of Kaduna State, Nigeria.

Muhammad Muhsin Fathuddin<sup>1\*</sup>, Saleh Alhaji Ado<sup>1</sup>, Muhammad Bashir Tijjani<sup>1</sup>, Haruna Makonjuola Kazeem<sup>2</sup>, John Solomon Obidah<sup>3</sup>, Rafeedah Fathuddin<sup>4</sup>

<sup>1</sup> Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

<sup>2</sup> Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

<sup>3</sup> Department of Microbiology, Faculty of Life Sciences, Modibbo Adama University, Yola, Adamawa State, Nigeria

<sup>4</sup> School of Life Sciences (SLS), B.S. Abdur Rahman Crescent Institute of Science & Technology, Chennai, Tamil Nādu. India

\* Correspondence: muhsinfathuddin@gmail.com; Tel.: (+234-8085350964)

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- **Milk is the fluid secreted by female mammals for nourishing offspring.**
- **It comprises a mixture of complex chemical substances, including fat, protein, lactose, and some mineral matters in the colloidal state in the form of a true solution [1].**
- **Resistotyping involves grouping bacterial isolates based on resistance patterns to a set of randomly chosen antibiotics peculiar to specific strains by phenotypic methods.**
- **Antimicrobial resistance (AMR) is increasing and has posed a major public health concern, severely limiting therapeutic options in clinical settings [2].**



# Objective



An objective of this work was to determine the resistotyping of *Salmonella* spp. and *Staphylococcus aureus* isolated from milk and milk products (*Kindirimo*, *Nono*, and *Yogurt*) sold in Zaria and Sabon-gari.



## Study Area

The study area included two (2) local government areas (Soba-gari and Zaria) in Kaduna state.

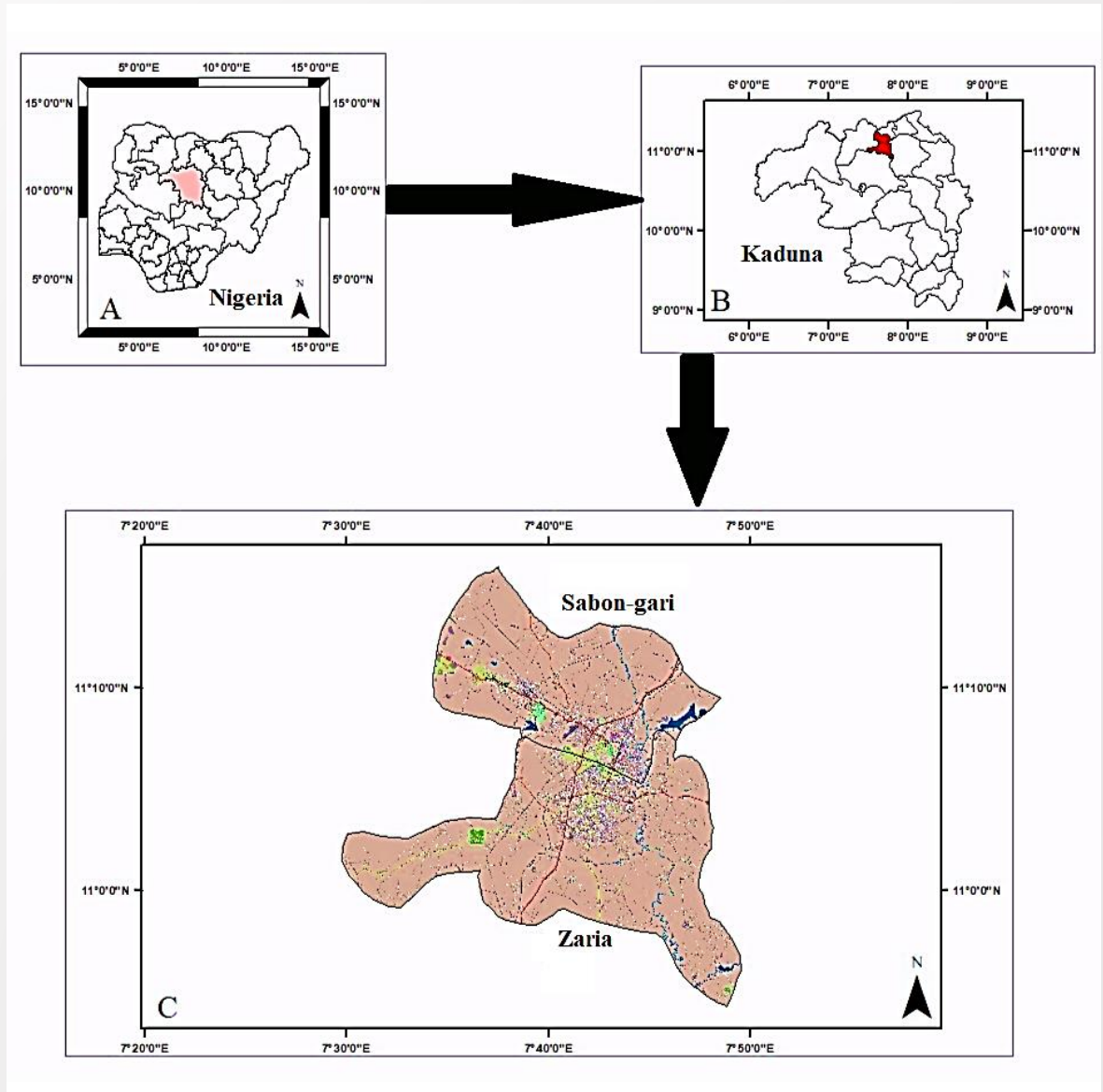


Figure 01: Map of Zaria and Sabon-gari



# Materials and Methods



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Selective Plating for *Staphylococcus (S.) aureus*: After incubation, a loopful of inoculum (from above) was inoculated on the Mannitol Salt Agar (MSA) plate and incubated at  $37\pm 1^{\circ}\text{C}$  for 30 hrs. and observed for growth (yellow halo) indicates *S. aureus*.





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Selective Plating for *Salmonella spp.*: To isolate *Salmonella spp.*, one (1) ml of pre-enrichment culture was inoculated into Rappaport-Vassiliadis Broth (RV) and incubated at  $42\pm 1^{\circ}\text{C}$  for 7 days. After the incubation, it was further cultured on selective agar plates of Salmonella Shigella Agar (SSA) at  $37^{\circ}\text{C}$  for 48 hrs.

## Antimicrobial Susceptibility Test (AST)/Resistotyping

- The AST for Ampicillin (AMP), Ciprofloxacin (CIP), Gentamicin (GEN), and Tetracycline (TET) were performed concurrently with Disc Diffusion Test (Bioanalyse, Turkey) using AM30 $\mu$ g, CIP05 $\mu$ g, GM10 $\mu$ g, and TE10 $\mu$ g concentrations, respectively, and E-Test (HiMedia, India) using 0.016-256  $\mu$ g/ml concentrations [3].
- Freshly prepared Mueller-Hinton Agar plates (HiMedia, India) were used for each test.
- The plates were inoculated with standardized inoculum (0.5 McFarland Standard) of isolates and incubated at 37°C for 24 hours.
- The Minimum Inhibitory Concentration (MIC) and Zone of Inhibition (ZOI) values of AMP, CIP, GEN, and TET
- Results are interpreted as S (Susceptible), I (Intermediate), or R (Resistant) based on the breakpoints
- Indicated by the reference standards for Clinical and Laboratory Standards Institute (CLSI) on <http://em100.edaptivedocs.net> [4] and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) on <http://www.eucast.org> [5].







# Results



## Organisms Isolated

A total of 122 isolates; *Salmonella* spp. (65) and *S. aureus* (57) were isolated from the samples

Sample	Milk		Kindirimo		Nono		Yoghurt		Total	
Organism	#	%	#	%	#	%	#	%	#	%
<i>Salmonella</i> spp.	24	36.92	20	30.77	18	27.69	03	04.62	65	100.00
<i>S. aureus</i>	23	38.60	18	31.58	12	21.05	05	08.77	57	100.00



# Results



Table 1. AST Summary

Group	Antimicrobial Resistance Pattern (Resistotyping)	Number Of Antibiotics	MAR Index	Number Of Isolates	Percent	Organism (Percent)
A	AMP-CIP-GEN-TET	4	1.00	000	00.000	—
B	AMP-CIP-GEN-TET	3	0.75	000	00.000	—
C	AMP-CIP-GEN-TET	3	0.75	000	00.000	—
D	AMP-CIP-GEN-TET	3	0.75	000	00.000	—
E	AMP-CIP-GEN-TET	3	0.75	000	00.000	—
F	AMP-CIP-GEN-TET	2	0.50	000	00.000	—
G	AMP-CIP-GEN-TET	2	0.50	000	00.000	—
H	AMP-CIP-GEN-TET	2	0.50	000	00.000	—
I	AMP-CIP-GEN-TET	2	0.50	036	29.508	<i>Salmonella</i> spp. (91.7), <i>S. aureus</i> (08.3)
J	AMP-CIP-GEN-TET	2	0.50	000	00.000	—
K	AMP-CIP-GEN-TET	1	0.50	000	00.000	—
L	AMP-CIP-GEN-TET	1	0.25	003	02.459	<i>Salmonella</i> spp. (33.3), <i>S. aureus</i> (66.7)
M	AMP-CIP-GEN-TET	1	0.25	000	00.000	—
N	AMP-CIP-GEN-TET	1	0.25	000	00.000	—
O	AMP-CIP-GEN-TET	1	0.25	044	36.066	<i>Salmonella</i> spp. (50.0), <i>S. aureus</i> (50.0)
P	AMP-CIP-GEN-TET	0	0.00	039	31.967	<i>Salmonella</i> spp. (23.1), <i>S. aureus</i> (76.9)
Total	083-122-122-042			122	100.000	

\* Key: Green – Susceptible ( $\geq 6$  mm), Red – Resistance ( $\leq 5$  mm)

Resistotyping of *Salmonella* spp. and *Staphylococcus aureus* from milk and milk products sold in Sabon-gari and Zaria Local Government Areas of Kaduna State, Nigeria.





# Results



**Table 2. AST Summary (CSLI)**

Group	Antimicrobial Resistance Pattern (Resistotyping)	Number Of Antibiotics	MAR Index	Number Of Isolates	Percent	Organism (Percent)
A	AMP-CIP-GEN-TET	4	1.00	003	02.459	<i>Salmonella</i> spp. (100.0)
B	AMP-CIP-GEN-TET	3	0.75	000	00.000	–
C	AMP-CIP-GEN-TET	3	0.75	001	00.820	<i>Salmonella</i> spp. (100.0)
D	AMP-CIP-GEN-TET	3	0.75	006	04.918	<i>Salmonella</i> spp. (100.0)
E	AMP-CIP-GEN-TET	3	0.75	000	00.000	–
F	AMP-CIP-GEN-TET	2	0.50	000	00.000	–
G	AMP-CIP-GEN-TET	2	0.50	002	01.639	<i>Salmonella</i> spp. (50.0), <i>S. aureus</i> (50.0)
H	AMP-CIP-GEN-TET	2	0.50	000	00.000	–
I	AMP-CIP-GEN-TET	2	0.50	061	50.000	<i>Salmonella</i> spp. (67.2), <i>S. aureus</i> (32.8)
J	AMP-CIP-GEN-TET	2	0.50	001	00.820	<i>S. aureus</i> (100.0)
K	AMP-CIP-GEN-TET	1	0.50	000	00.000	–
L	AMP-CIP-GEN-TET	1	0.25	007	05.738	<i>Salmonella</i> spp. (28.6), <i>S. aureus</i> (71.4)
M	AMP-CIP-GEN-TET	1	0.25	001	00.820	<i>S. aureus</i> (100.0)
N	AMP-CIP-GEN-TET	1	0.25	003	02.459	<i>Salmonella</i> spp. (33.3,) <i>S. aureus</i> (66.7)
O	AMP-CIP-GEN-TET	1	0.25	027	22.131	<i>Salmonella</i> spp. (33.7), <i>S. aureus</i> (66.7)
P	AMP-CIP-GEN-TET	0	0.00	010	08.197	<i>Salmonella</i> spp. (10.0), <i>S. aureus</i> (90.0)
Total	041-108-116-018			122	100.000	

\* Key: **Green** – Susceptible, **Red** – Resistance

*Resistotyping of Salmonella spp. and Staphylococcus aureus from milk and milk products sold in Sabon-gari and Zaria Local Government Areas of Kaduna State, Nigeria.*



Table 3. AST Summary (EUCAST)

Group	Antimicrobial Resistance Pattern (Resistotyping)	Number Of Antibiotics	MAR Index	Number Of Isolates	Percent	Organism (Percent)
A	AMP-CIP-GEN-TET	4	1.00	012	09.836	<i>Salmonella</i> spp. (41.7), <i>S. aureus</i> (58.3)
B	AMP-CIP-GEN-TET	3	0.75	002	01.639	<i>Salmonella</i> spp. (100.0)
C	AMP-CIP-GEN-TET	3	0.75	005	04.098	<i>S. aureus</i> (100.0)
D	AMP-CIP-GEN-TET	3	0.75	070	57.377	<i>Salmonella</i> spp. (70.0), <i>S. aureus</i> (30.0)
E	AMP-CIP-GEN-TET	3	0.75	000	00.000	—
F	AMP-CIP-GEN-TET	2	0.50	000	00.000	—
G	AMP-CIP-GEN-TET	2	0.50	009	07.377	<i>Salmonella</i> spp. (100.0)
H	AMP-CIP-GEN-TET	2	0.50	000	00.000	—
I	AMP-CIP-GEN-TET	2	0.50	024	19.672	<i>S. aureus</i> (100.0)
J	AMP-CIP-GEN-TET	2	0.50	000	00.000	—
K	AMP-CIP-GEN-TET	1	0.50	000	00.000	—
L	AMP-CIP-GEN-TET	1	0.25	000	00.000	—
M	AMP-CIP-GEN-TET	1	0.25	000	00.000	—
N	AMP-CIP-GEN-TET	1	0.25	000	00.000	—
O	AMP-CIP-GEN-TET	1	0.25	000	00.000	—
P	AMP-CIP-GEN-TET	0	0.00	000	00.000	—
Total	011-029-103-000			122	100.000	

\* Key: Green – Susceptible, Red – Resistance

Resistotyping of *Salmonella* spp. and *Staphylococcus aureus* from milk and milk products sold in Sabon-gari and Zaria Local Government Areas of Kaduna State, Nigeria.



Table 4. E-Test Summary

Organism	Antibiotic strip (Abb.)	Concentration [µg/ml]	CLSI breakpoints (MIC) [µg/ml]			Number of isolates*		
			S	I	R	S	I	R
<i>Salmonella</i> spp.	Ampicillin (AMP)	0.016-256	≤08	16	≥32	06	–	02
	Ciprofloxacin (CIP)	0.016-256	≤0.06	0.12 - 0.5	≥01	02	02	04
	Gentamicin (GEN)	0.016-256	≤02	04	≥08	08	–	–
	Tetracycline (TET)	0.016-256	≤04	08	≥16	03	02	03
<i>S. aureus</i>	Ampicillin (AMP)	0.016-256	≤04	–	≥08	04	–	04
	Ciprofloxacin (CIP)	0.016-256	≤01	02	≥04	06	02	02
	Gentamicin (GEN)	0.016-256	≤04	08	≥16	05	01	01
	Tetracycline (TET)	0.016-256	≤04	08	≥16	03	02	03



# Discussion



- The study conducted by Tamba et al., 2016 [6] on *Salmonella* isolates showed resistance rates AMP (85.7%), TET (35.7%), CIP (00.0%), and GEN (00.0%). Their findings indicated that AMP is the most resistant drug.
- Our study shows there is an increase in resistance rates among *Salmonella* isolates on all the other drugs tested, TET had (95.385%) on CLSI breakpoint only, CIP (15.385%), and GEN (04.615%) on both CLSI and EUCAST.
- While AMP showed a drop from 85.7% to 81.538%, TET had an alarming jump from 35.7% to 95.385%.





# Discussion



- In the case of *S. aureus* isolates, Umaru et al., 2013 [7] used the CIP, GEN, and TET along with others, the resistance rate was TET (55.5%), CIP (38.9%), GEN (11.1%), and oxacillin (100.0%) which can be substituted for AMP [4], thus, making AMP, the most resistance drug in that study.
- This study shows there is an increase in resistance rates among *S. aureus* isolates on all the drugs tested; TET on the CLSI (75.439%), and on EUCAST (100.000%), AMP (52.632%) CLSI only, CIP on the CLSI (10.526%), on EUCAST (43.860%), and GEN on CLSI (10.526%) and on EUCAST (43.860%). The same pattern is observed here an alarming jump in TET (55.5% to 75.439%) and a drop in AMP (100.0% to 52.632%).
- From Table 4, half of the selected isolates are resistant to AMP and TET, with all being susceptible to CIP and GEN. However, in Table 2, AMP in *Salmonella* spp. was 25%R, and *S. aureus* was 50%R, TET was 37.5%R for both, CIP was 50%R and 25%R respectively, GEN was 00%R and 12.5% R respectively.



# Conclusion



- In summary, recent research has revealed a surge in antibiotic resistance, particularly in TET, which is the most resistant drug for both organisms. AMP, CIP, and GEN follow in that sequence.
- It is imperative that prompt measures are taken to tackle the escalating issue of antimicrobial resistance (AMR) and curb the proliferation of antibiotic-resistant pathogens.



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