

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.

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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.

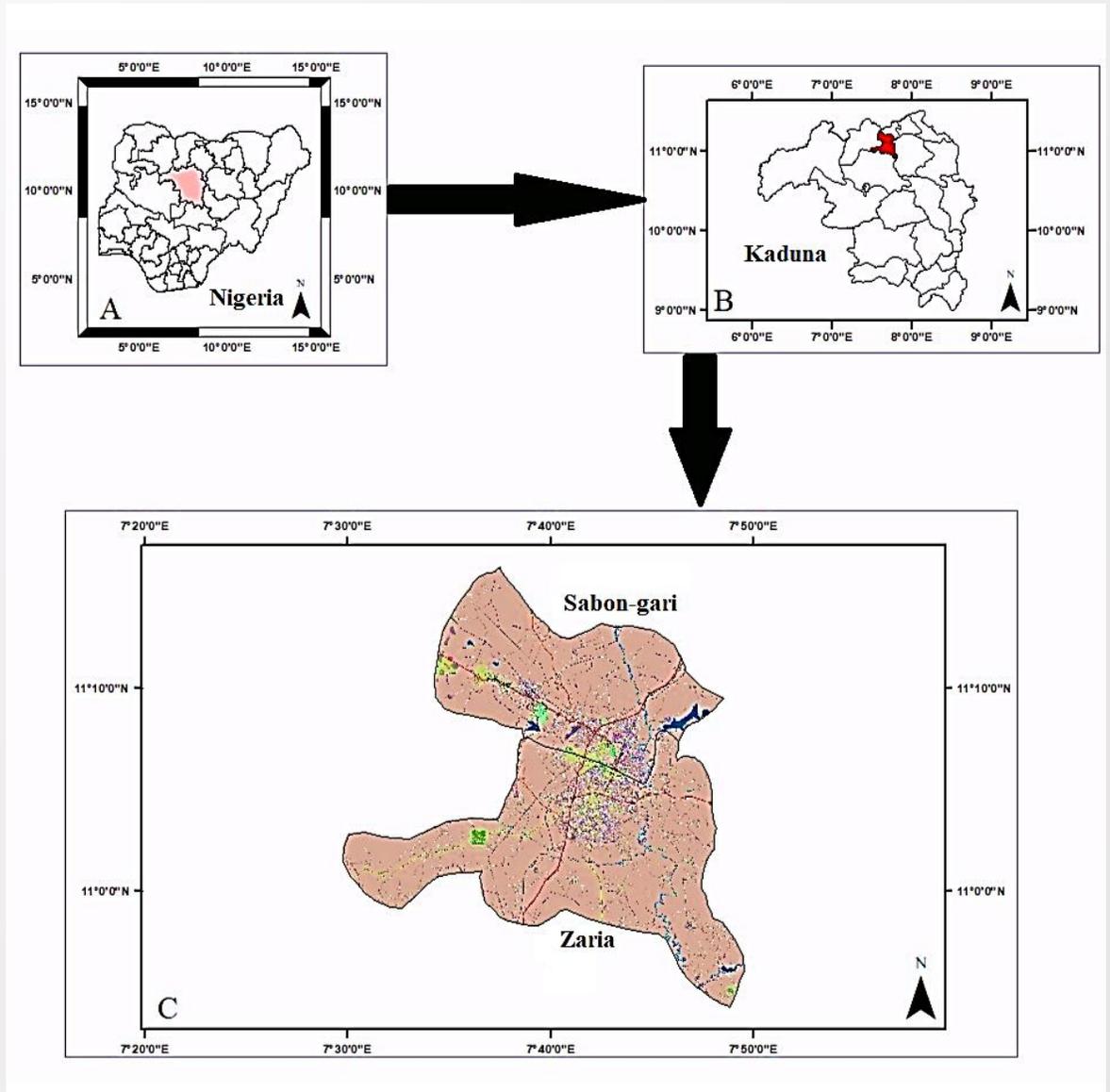


Figure 01: Map of Zaria and Sabon-gari



Materials and Methods



ISOLATION OF ORGANISMS





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Four hundred (400) samples [100 each] of milk and milk products (Kindirimo, Nono, and Yogurt) sold in Zaria and Sabon-gari.



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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°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 μ g, CIP05 μ g, GM10 μ g, and TE10 μ g concentrations, respectively, and E-Test (HiMedia, India) using 0.016-256 μ 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	
	#	%	#	%	#	%	#	%	#	%
<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 (≥ 6 mm), **Red** – Resistance ($= 5$ 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



Results



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

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



- 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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Thank
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