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EFFICACY OF DISINFECTANTS USAGE AT DAILY LIVE BIRD MARKETS IN FOUR NORTH-WESTERN STATES OF NIGERIA

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ABSTRACT

Maintaining strict biosecurity measures are essential in preventing disease spread from live bird markets (LBMs), which serve as a major intermingling area for poultry from different sources. This study evaluated the efficacy of disinfectants used in daily live bird markets of four north-western states in Nigeria. Seven different disinfectants were identified as commonly used in the LBMs. They were analysed by suspension and surface disinfection tests against standard strains of *Escherichia coli*, *Salmonella Enteritidis*, and *Staphylococcus aureus*. Isolates from swab samples of birds' cages in the LBMs were initially subjected to biochemical tests and, subsequently, susceptibility tests against commercial disinfectants. All of the 7 (100 %) disinfectants used in the LBMs killed/inhibited the growth of *E. coli*, *S. Enteritidis*, and *S. aureus* with the suspension test, while following the surface disinfection test, all 7 (100 %) killed/inhibited the growth of *E. coli* and S. *Enteritidis* but only 4 (57 %) killed/inhibited the growth of *S. aureus*. Seven (0.02 %) samples out of the 400 swabs were positive for *E. coli* comprising 1 (14 %), 2 (29 %), and 4 (57 %) from LBMs in Katsina, Kaduna, and Kano, respectively. There were varying growths of *E. coli* at different concentrations and exposure times. Six (17 %) of the LBMs sampled had and used disinfectants. *E. coli* was isolated from 1 (17 %) out of the 6 LBMs that had and used disinfectants and 5 (17 %) out of the 29 LBMs that did not have or use disinfectants. The standard organisms were most susceptible to orthobenzyl chlorophenol-based disinfectants and least susceptible to chlorophenol-based disinfectants. This study has shown the importance of the use of disinfectants in LBMs. There should be enforcement of disinfectants usage in LBMs for public safety.

Key words: biosecurity; efficacy; disinfectants; live bird markets; North-western Nigeria

INTRODUCTION

The transmission of diseases through surfaces plays an essential role in spreading infection among humans and other animals. The survival of pathogens such as viruses, bacteria, fungi, etc. on surfaces can have a direct impact on preventive measures, including hygiene guidelines and disinfection strategies [29]. Disinfection is the killing/inhibition of microorganisms, not necessarily destruction but

reducing to a lower acceptable level of microbial activity. Hence, a termination of the chain of bugs transmission to avoid infections [17]. Disinfection is an integral part of preventive medicine, which cuts off infection routes. However, the effectiveness of disinfection depends on selecting disinfectants according to the situation and environment, which usually requires expertise and experience. Several properties of an ideal disinfectant and five critical considerations for selecting the optimal disinfectant have been highlighted. Fundamental properties of a good disinfectant have been described by R u t a l a and W e b e r [24]. It is essential to make use of a disinfectant that has the majority of these important properties, and it is advisable to select a disinfectant with a longer contact time to kill a broader spectrum of microorganisms. The growth rate of disinfectant-resistant bacteria is shocking, which will greatly reduce the killing efficiency of disinfectants [31]. Disinfectant resistance is related to the bacterial characteristics, concentration and physical state of disinfectants and environmental conditions [28]. High-level disinfection will inactivate all microorganisms except large numbers of bacterial spores [23]. Intermediate-level disinfection will inactivate vegetative microorganisms and possibly low numbers of bacterial spores. Low-level disinfection inactivates most vegetative bacteria and some fungi and viruses, but it does not inactivate bacterial spores. Alcohol is an excellent example of a low-level disinfectant, with a relatively broad spectrum for microorganisms but is slow to act against non-enveloped viruses. Additionally, alcohol is not a good choice to achieve coverage of the necessary contact period with microorganisms because of its volatility, and it is not recommended for use on large surfaces such as cages of live bird markets because of its flammability. Generally, the contact period should be longer than or equal to the kill time. Several disinfectants that contain different classes of chemical compounds, including acids, alcohols, aldehydes, alkalis, biguanides, halogens, oxidizing agents, phenols, and quaternary ammonium compounds as active substances have been developed and proposed for practical use, and they are recommended for use in different branches of industry [22].

Live bird markets (LBMs) are also known as wet markets and are common in many parts of the World [10], including Nigeria. There were no dedicated live bird markets (LBMs) and poultry processing facilities in Nigeria until recently. The majority of the processing facilities are also devoid of formal dedicated Veterinary Inspectors, thus lacking expertise in dilution/preparation of disinfectants. Hence, there is a need to evaluate overall live bird market operations to assess the country's public health problems related to poultry processing [19]. In some selected urban LBMs, the marketers have been trained and educated on the dangers of highly pathogenic avian influenza (HPAI) to poultry and humans [3] and were taught the disinfection procedures, but the impact of these measures on the safety of poultry products and the control of other zoonoses were never evaluated. In Nigeria, there is also a problem with the quality of drugs and chemicals, where producers may use a lesser amount of the active ingredient required for it to be effective. In addition, the live bird marketers are generally uneducated, which makes it difficult for them to follow manufacturers' instructions strictly, leading to improper use of these chemicals. These will consequently promote resistance and the failure of disinfection.

Infected poultry can contaminate the LBM environment, such as swabs from floors, cages, drains, walls, scales, and microorganisms may persist for days or weeks depending on factors such as humidity, temperature, and the presence of several host ranges [12]. There is a dearth of information on disinfectants' efficacy in Nigeria's LBMs. This study will generate information on the efficacy of disinfectants that can guide LBM operators (and other relevant authorities) on the correct use of disinfectants in LBMs. It will, in turn, reduce the likelihood of the development of resistance and the spread of pathogens during poultry handling and processing and hence reduce human exposure to poultry zoonoses. It will reduce the cost of treatment in both poultry and humans, which will positively impact the economy.

This study aimed to identify the type(s) of disinfectant(s) used in the LBMs and determine their efficacy against reference strains of *Escherichia coli*, *Salmonella Enteritidis*, and *Staphylococcus aureus*. Also, to determine the *in vitro* antibacterial activity of the identified disinfectants.

MATERIALS AND METHODS

Study area

The study was conducted at the daily live bird markets in some North-western states (Kaduna, Kano, Katsina, and Zamfara) of Nigeria. Northwest is one of the six geopolit-

Fig. 1. Map of North-western Nigeria
(Source; Google images)

ical zones in Nigeria and consists of seven (7) states: Jigawa, Kaduna, Kano, Katsina, Kebbi, Sokoto, and Zamfara states (Fig. 1).

Study design

The study design was experimental, and it was divided into two parts. The first part was conducted to test the efficacy of routinely used disinfectants in daily LBMs of the states against standard control organisms. *Escherichia coli* isolated from swab samples taken from the LBMs were subjected to a susceptibility test using some commercial disinfectants for the second experiment.

Sampling techniques

Disinfectant samples from managers were collected based on availability. The swab sample size collected from Marketers was determined using the formula by Thrusfield [27]. The prevalence rate of 56% [22] was used to obtain the sample size was 379. In the present study, 5.5% of the calculated sample size was added to minimize loss due to sampling error. Hence, 400 swab samples were collected from the bird's cages of the marketers.

Live bird markets within the metropolitan area of these states were identified. Disinfectant (10 ml each) and swab samples were collected from managers based on convenience and availability. The number of swab samples taken from each state was calculated by proportionate sampling from which Kaduna (with 12 LBMs) = 137, Kano (15) = 171, Katsina (4) = 46, and Zamfara (4) = 46. Swab samples of birds' cages (a single swab from each marketer) of about 10 cm^2 of the innermost area of the floor of the birds' cages were collected using a sterile swab stick, and this was transferred into transport media and taken to bacteria laboratory for isolation.

Testing of disinfectant samples

It involved a standard laboratory protocol according to the European Committee for Standardization guideline, where the basic principle for testing the efficacy of a disinfectant is in three phases; first, testing for its antimicrobial activity; second, testing the condition and in-use dilution at which it is active; and lastly an in situ test [21]. The first two phases were carried out in this study.

Suspension test

Standard strains of reference *Escherichia coli* (ATCC 10536; NCTC 10418), *Salmonella Enteritidis* (ATCC 13076; NCTC 12694), and *Staphylococcus aureus* (ATCC 6538; NCTC10788), were grown on media. Nutrient broth cultures of these reference bacteria were prepared in a test tube to make a 10^4 dilution of the bacteria.

A loopful (10 µl) of the suspension was taken and brought into contact with 2 ml of the disinfectant in a second test tube. A loopful (10 µl) of the resultant mixture was inoculated onto a culture medium. Eosin methylene blue (EMB) was used for *Escherichia coli*; eoxycholate citrate agar (DCA)/*Salmonella Shigella* agar (SSA) for *Salmonella Enteritidis* and Mannitol salt agar (MSA) for *Staphylococcus aureus*. A control was included for each category. These were incubated at 37°C for 24 h after which they were observed for growth. Growth was recorded as positive (+), whereas its absence was negative (–).

Surface disinfection test

The disinfectant samples collected from the LBMs were prepared by diluting according to the working dilution of the LBMs. There were prepared 2-fold and 4-fold dilutions to achieve three different concentrations. A microscopic glass slide (serving as the test surface) was contaminated with 0.2 ml of a 10^4 dilution of a nutrient broth culture of the test bacteria. The slides were dried and placed on a rack. One ml each of the disinfectant was dropped on the slides using a pipette and distributed evenly. A control was included with the disinfectant substituted with sterile distilled water. These were left to stand for different intervals (5, 15, and 30 minutes), after which the slides were rinsed with 50 ml of distilled water. The rinsing

fluids were inoculated on a culture medium and incubated at 37°C for 24—48 h.

Isolation of *E. coli*

One ml of the swabbed sample was taken and added to 9 ml of tryptone soy broth as enrichment and incubated for 24 hours. This was then plated on a selective media (EMB) and incubated for 24 h as first described by L e v i n e [16]. Suspected positive colonies (with characteristic greenish metallic sheen on *E. coli*) were transferred into nutrient broth in sterile sample bottles and afterward subjected to conventional biochemical and kit (Microbact™) tests.

Susceptibility test

The isolated *Escherichia coli* from LBMs were subjected to susceptibility tests against commercially available disinfectants. Four commercially available disinfectants were used, referred to here as A, B, C, and D. A contains ortho-benzyl chlorophenol, B contains chloroxylenol, C contains chlorophenol, and D contains sodium hypochlorite as active ingredients. The maximum concentration was the available commercial concentration from the manufacturers. These concentrations were diluted with sterile distilled water to obtain 1.28%, 0.32%, 0.08%, and 0.02% for the respective disinfectant.

A: 0.02, 0.08, 0.32, 1.28 and 5.12%

B: 0.02, 0.08, 0.32, 1.28 and 4.80%

C: 0.02, 0.08, 0.32, 1.28 and 5.12%

D: 0.02, 0.08, 0.32, 1.28 and 3.50%

The isolates were standardized to 1% McFarland in normal saline, inoculated into the nutrient broth, and incubated at 37°C for 24 h.

A half ml of the 24 h culture was added to 5 ml of the different concentrations of disinfectant solutions, placed in a water bath (at 19°C), and shaken gently. These were removed at different time intervals (5, 10, 15, 30, and 60 min). One loopful of the mixture was then sub-cultured into 5 ml of nutrient broth and incubated at 37°C for 48—72 hrs.

The results were recorded as positive when there was an increase in turbidity or negative when there was none when compared to the controls. These were quantified and also presented as percentages.

RESULTS

Results of the suspension, surface disinfection, and susceptibility tests are presented in tables.

Types of disinfectants used in the LBMs and their efficacy

Table 1 shows LBMs that had disinfectant samples, the name of the disinfectant, form, chemical composition, and the dilution factor used in the LBM.

The characteristic growth of the organisms was obtained when inoculated onto individual selective media. *E. coli* on eosin-methylene blue (EMB) appeared as dark,

Table 1. Disinfectants used in some LBMs of north-western Nigeria

	State	LBM	Disinfectant	Form	Chemical composition
1	Katsina	Sabuwar kofa	Diskol I	Liquid	Glutaraldehyde, ammonium chloride, formaldehyde
2	Katsina	Central market	Lysol	Liquid	Methylphenol
3	Kano	Sharada	Virkon-S I	Powder	Potassium peroxymonosulphate, sodium dodecylbenzene sulfonate, sulphamic acid
4	Kano	Sharada	Diskol II	Liquid	Glutaraldehyde, ammonium chloride, formaldehyde
5	Kano	Sheka	Hypo	Liquid	Sodium hypochlorite
6	Kano	Sabon titi	Vinkokill	Liquid	Chlorophenol
7	Kaduna	Sokoto road	Virkon-S II	Powder	Peroxymonosulphate

S/N—Serial Number

Table 2. The activity of some disinfectants used in live bird markets in north-western Nigeria on *Staphylococcus aureus*

Time	Dilution	C	Diskol I	Disinfectants			Hypo	Vinkokill	Virkon-S II
				Lysol	Virkon-S I	Diskol II			
	Market	+	–	–	–	–	–	–	–
5 min	2-fold	+	+1	–	–	–	+4	–	+1
	4-fold	+	+3	–	–	+1	+5	–	+7
	Market	+	–	–	–	–	–	–	–
15 min	2-fold	+	–	–	–	–	–	–	–
	4-fold	+	–	–	–	–	–	–	–
	Market	+	–	–	–	–	–	–	–
30 min	2-fold	+	–	–	–	–	–	–	–
	4-fold	+	–	–	–	–	–	–	–

blue-black colonies with a greenish metallic sheen. *Salmonella Enteritidis* on desoxycholate agar (DCA) as pale/colourless colonies with dark centres due to hydrogen sulphide production. *Staphylococcus aureus* on mannitol salt agar (MSA) as yellow colonies with yellow zones.

Following the suspension test, no growth was observed in all inoculates except controls.

All the disinfectants inhibited the growth of *Escherichia coli* at all three dilutions and the different periods of exposure. Growths were only seen in the controls. There was no growth at the three dilutions and at different exposure times when *Salmonella Enteritidis* was exposed to disinfectants. Growth was also seen in the controls. Table 2 shows the action of the disinfectants on *Staphylococcus aureus* at different time intervals (of exposure) and different concentrations following the surface disinfection test. For *Staphylococcus aureus*, there was growth in all of the controls. Growth was recorded after exposure to disinfectants 1, 4, 5, and 7. The growth was seen at 5 min exposure time and at second and third dilution except for disinfectant 4 where growth was only seen at the third dilution. The number of colonies increased with increasing dilution.

In vitro antibacterial activity of some commercial disinfectants

Out of the total of 400 swab samples collected, 121 produced the characteristic "blue-black" colonies with greenish metallic sheen on eosin-methylene blue following the culture and isolation for *E. coli*. These were the suspect positives.

Following the MR/VP, citrate, urease, SIM, and TSI tests, and the kit (Microbact™) tests on the 121 suspect positive samples, 7 samples were positive for *E. coli*. These were urease negative, citrate negative, indole positive, motile, hydrogen sulphide negative, methylene red positive, and Vvoges Proskauer negative, and showed percentage probability (of being *E. coli*) from the ID system of 97.11, 91.92, 84.29, 97.11, 97.11, 92.97 and 92.11% comprising of 1 from an LBM in Katsina, 2 from Kaduna and 4 from Kano (KT 01 13, KN 05 01, KN 06 03, KN 09 03, KN 13 03, KD 06 03 and KD 11 01. Where the first number represent the LBM and the second number represent the sample number).

The action of each disinfectant on individual isolates at varying concentrations and at different exposure times was observed as the presence of growth (+) or no growth (–) and presented as tables of concentration (horizontal axis) against periods of exposure (vertical axis) (Tables 3, 4, 5, and 6).

The activity of orthobenzyl chlorophenol on *Escherichia coli*

Two isolates, one from Kano (KN 06 03) and the second from Kaduna (KD 06 03), were most susceptible as growth was observed only at the least concentration of 0.02% and an exposure time of 5 minutes. Another from Kano was a bit more resistant, with growth at the next concentration of 0.08 %. The least susceptible was an isolate from Kaduna (KD 11 01), with growth observed at a concentration of 1.28%, and an isolate from Kano (KN 13 03),

Table 3. The activity of orthobenzyl chlorophenol on *Escherichia coli*

E. coli isolate	Conc. [%]	Duration of exposure [min]					*E. coli* isolate	Conc. [%]	Duration of exposure [min]				
		5	10	15	30	60			5	10	15	30	60
KT 01 13	5.12	–	–	–	–	–	KN 05 01	5.12	–	–	–	–	–
	1.28	–	–	–	–	–		1.28	–	–	–	–	–
	0.32	–	–	–	–	–		0.32	–	–	–	–	–
	0.08	+	+	–	–	–		0.08	+	–	–	–	–
	0.02	+	+	–	–	–		0.02	+	–	–	–	–
KN 06 03	5.12	–	–	–	–	–	KN 09 03	5.12	–	–	–	–	–
	1.28	–	–	–	–	–		1.28	–	–	–	–	–
	0.32	–	–	–	–	–		0.32	+	–	–	–	–
	0.08	–	–	–	–	–		0.08	+	–	–	–	–
	0.02	+	–	–	–	–		0.02	+	+	–	–	–
KN 13 03	5.12	–	–	–	–	–	KD 06 03	5.12	–	–	–	–	–
	1.28	–	–	–	–	–		1.28	–	–	–	–	–
	0.32	+	–	–	–	–		0.32	–	–	–	–	–
	0.08	+	+	–	–	–		0.08	–	–	–	–	–
	0.02	+	+	+	–	–		0.02	+	–	–	–	–
KD 11 01	5.12	–	–	–	–	–							
	1.28	+	–	–	–	–							
	0.32	+	–	–	–	–							
	0.08	+	–	–	–	–							
	0.02	+	+	–	–	–							

with growth observed at an exposure time of 15 minutes (Table 3). The percentage growth was recorded as 16, 8, 4, 16, 24, 4 and 20 % for *E. coli* isolates KT 01 13, KN 05 01, KN 06 03, KN 09 03, KN 13 03, KD 06 03 and KD 11 01, respectively.

The activity of chloroxylenol on *Escherichia coli*

The most susceptible was an isolate from Kano (KN 09 03), with growth observed only at a concentration of 0.02 % with a 5 min exposure time. The least susceptibility was observed in an isolate from Kano (KN 13 03), with growth even at concentrations of 1.28 %, 0.32 % at 10 min, and with an exposure time of 15 min (Table 4). The percentage growth was recorded as 28, 32, 12, 4, 32, 20 and 20 % for *E. coli* isolates KT 01 13, KN 05 01, KN 06 03, KN 09 03, KN 13 03, KD 06 03 and KD 11 01, respectively.

The activity of chlorophenol on *Escherichia coli*

An isolate from Kano (KN 05 01) and two from Kaduna (KD 06 03 and KD 11 01) were least susceptible to the disinfectant even at the highest concentration of 5.12 %. The second from Kaduna (KD 11 01) was resistant to the disinfectant at the highest exposure time of 60 minutes. The highest susceptibility was observed with an isolate from Kano (KN 06 03) with inhibition of growth from the concentration of 0.32 % (Table 5). The percentage growth was recorded as 60, 36, 28, 28, 56, 56 and 52 % for *E. coli* isolates KT 01 13, KN 05 01, KN 06 03, KN 09 03, KN 13 03, KD 06 03 and KD 11 01, respectively.

The activity of sodium hypochlorite on *Escherichia coli*

The least susceptibility was observed with the isolate from Katsina (KT 01 13), with observed growth at

Table 4. The activity of chloroxylenol on *Escherichia coli*

E. coli isolate	Conc. [%]	Duration of exposure [min]					*E. coli* isolate	Conc. [%]	Duration of exposure [min]				
		5	10	15	30	60			5	10	15	30	60
KT 01 13	4.80	–	–	–	–	–	KN 05 01	4.80	–	–	–	–	–
	1.28	–	–	–	–	–		1.28	–	–	–	–	–
	0.32	+	+	–	–	–		0.32	+	+	–	–	–
	0.08	+	+	–	–	–		0.08	+	+	–	–	–
	0.02	+	+	+	–	–		0.02	+	+	+	+	–
KN 06 03	4.80	–	–	–	–	–	KN 09 03	4.80	–	–	–	–	–
	1.28	–	–	–	–	–		1.28	–	–	–	–	–
	0.32	–	–	–	–	–		0.32	–	–	–	–	–
	0.08	+	–	–	–	–		0.08	–	–	–	–	–
	0.02	+	+	–	–	–		0.02	+	–	–	–	–
KN 13 03	4.80	–	–	–	–	–	KD 06 03	4.80	–	–	–	–	–
	1.28	+	–	–	–	–		1.28	–	–	–	–	–
	0.32	+	+	–	–	–		0.32	+	–	–	–	–
	0.08	+	+	–	–	–		0.08	+	–	–	–	–
	0.02	+	+	+	–	–		0.02	+	+	+	–	–
KD 11 01	4.80	–	–	–	–	–							
	1.28	–	–	–	–	–							
	0.32	+	–	–	–	–							
	0.08	+	–	–	–	–							
	0.02	+	+	+	–	–							

a concentration of 3.5% at both exposure times of 5 and 10 minutes. The highest susceptibility was observed with an isolate from Kano (KN 09 03), with growth observed at a concentration of 0.32% (Table 6). The percentage growth was recorded as 48, 24, 40, 12, 12, 44 and 32% for *E. coli* isolates KT 01 13, KN 05 01, KN 06 03, KN 09 03, KN 13 03, KD 06 03 and KD 11 01, respectively.

DISCUSSION

From our study, the calculated prevalence as regards to the use of disinfectants in LBMs was 17% which was close to the 23% reported in Bangladesh [7]. It is far lower than the 56% prevalence of FAO in 2008 [9] but higher than the 4.5% disinfectant usage reported in Malaysia [16]. The high prevalence reported by FAO could be attributed to the rigorous control campaign with the call for the use of disinfectants in LBMs following the avian influenza outbreaks between 2006 and 2009. The stakeholders have since been growing more complacent about disinfection in LBMs, which has led to the decline.

Following the suspension test, the absence of growth in all reactions showed that all of the disinfectants could kill/inhibit the growth of the bacteria they were tested against. However, the suspension test alone cannot be used to justify the efficacy claim of the disinfectants or the susceptibility of the bacteria because it does not represent the effectiveness of the disinfectant in the actual environment where the bacteria are attached to a specific material.

Despite resistant *E. coli* and *Salmonella* spp. have been reported to be prevalent and associated with older farms

Table 5. The activity of chlorophenol on *Escherichia coli*

E. coli isolate	Conc. [%]	Duration of exposure [min]					*E. coli* isolate	Conc. [%]	Duration of exposure [min]				
		5	10	15	30	60			5	10	15	30	60
KT 01 13	5.12	–	–	–	–	–	KN 05 01	5.12	+	–	–	–	–
	1.28	+	+	+	–	–		1.28	+	–	–	–	–
	0.32	+	+	+	–	–		0.32	+	–	–	–	–
	0.08	+	+	+	+	–		0.08	+	+	–	–	–
	0.02	+	+	+	+	+		0.02	+	+	+	+	+
KN 06 03	5.12	–	–	–	–	–	KN 09 03	5.12	–	–	–	–	–
	1.28	–	–	–	–	–		1.28	–	–	–	–	–
	0.32	–	–	–	–	–		0.32	+	+	–	–	–
	0.08	+	+	+	–	–		0.08	+	+	–	–	–
	0.02	+	+	+	+	+		0.02	+	+	+	–	–
KN 13 03	5.12	–	–	–	–	–	KD 06 03	5.12	+	+	–	–	–
	1.28	+	+	–	–	–		1.28	+	+	+	–	–
	0.32	+	+	–	–	–		0.32	+	+	+	–	–
	0.08	+	+	+	+	+		0.08	+	+	+	–	–
	0.02	+	+	+	+	+		0.02	+	+	+	–	–
KD 11 01	5.12	+	–	–	–	–							
	1.28	+	–	–	–	–							
	0.32	+	+	–	–	–							
	0.08	+	+	+	+	–							
	0.02	+	+	+	+	+							

and live bird markets [2, 4], the surface disinfection test in our study revealed that all of the disinfectants were active against *E. coli* and *S. Enteritidis*, even at the lowest concentration and least exposure time. A previous study reported a similar result in a response of *Escherichia coli* and *Salmonella Enteritidis* to disinfection, and it proposed the use of *E. coli* as an indicator for possible *Salmonella* presence after cleaning and disinfection [8]. In the case of *S. aureus*, growth was observed under only five minutes of exposure time for the various disinfectant with the exception of Lysol and Vinkokill. This may be due to the variation in time required for the mechanism of action of the respective disinfectants to manifest. Although, it has been demonstrated that complete inhibition can be obtained with a shorter exposure duration using a certain concentration of the disinfectant [6]. The differences in growth activity from *S. aureus* between similar disinfectants (Diskol I and II, Virkons I and II) may be due to the fact that they were sourced from different LBMs where they were subjected to different storage conditions and periods. Disinfects not stored at cool, dark, and appropriate covering usually result in instability and inactivation of the solution, rendering it less or ineffective [25].

Escherichia coli was isolated from the swab samples collected, comprising one from Katsina, two from Kaduna, and four from Kano. The results from the susceptibility testing showed varying reactions. From our study, the activity of orthobenzyl chlorophenol on the isolates shows that KN 13 03 and KD 11 01 had a higher percentage growth (24% and 20%, respectively) than the other isolates. This may be due to these LBMs being larger, more populated by humans and birds, and hence more activities.

Table 6. The activity of sodium hypochlorite on *Escherichia coli*

E. coli isolate	Conc. [%]	Duration of exposure [min]					*E. coli* isolate	Conc. [%]	Duration of exposure [min]				
		5	10	15	30	60			5	10	15	30	60
KT 01 13	3.50	+	+	–	–	–	KN 05 01	3.50	–	–	–	–	–
	1.28	+	+	–	–	–		1.28	–	–	–	–	–
	0.32	+	+	–	–	–		0.32	+	–	–	–	–
	0.08	+	+	+	–	–		0.08	+	+	–	–	–
	0.02	+	+	+	–	–		0.02	+	+	+	–	–
KN 06 03	3.50	–	–	–	–	–	KN 09 03	3.50	–	–	–	–	–
	1.28	–	–	–	–	–		1.28	–	–	–	–	–
	0.32	+	+	–	–	–		0.32	–	–	–	–	–
	0.08	+	+	+	–	–		0.08	+	–	–	–	–
	0.02	+	+	+	+	+		0.02	+	+	–	–	–
KN 13 03	3.50	–	–	–	–	–	KD 06 03	3.50	–	–	–	–	–
	1.28	–	–	–	–	–		1.28	+	–	–	–	–
	0.32	+	–	–	–	–		0.32	+	+	+	–	–
	0.08	+	–	–	–	–		0.08	+	+	+	–	–
	0.02	+	–	–	–	–		0.02	+	+	+	+	–
KD 11 01	3.50	–	–	–	–	–							
	1.28	–	–	–	–	–							
	0.32	+	–	–	–	–							
	0.08	+	+	–	–	–							
	0.02	+	+	+	+	+							

These huge activities will require the need to disinfect frequently, but the workers and managers do dilute the disinfectant below recommended dilution so as to cut costs. It has been reported that places with more human activity tend to have more antimicrobial-resistant *E. coli* [30]. Also, the level of contamination and spread of disease and resistant bacteria in LBM may be associated with the volume of trade, environment, and type of poultry at the market [15]. Our study revealed that orthobenzyl chlorophenol inhibits/kills the isolated *E. coli* completely from 15 minutes upward. Hence, LBM workers and managers need to use this disinfectant for an exposure duration greater than 15 minutes during cleaning and disinfection.

The least susceptible isolate to chloroxylenol was from KN 13 03, where growth was recorded at 15 minutes exposure time and at 1.28% concentration. Although chloroxylenol has been established to kill bacteria and enveloped viruses by compromising the membrane proteins responsible for transport and signalling. However, it shows some level of discrimination against bacteria, possibly due to the magnitude of changes in the bacteria membrane environment [20].

Virtually all of the isolates showed growth when exposed to chlorophenol at a lower concentration irrespective of the duration of the exposure. Hence, this indicates disinfection cannot be achieved with chlorophenol at a lower concentration even when exposed for a long period of time. This may be due to chlorophenols being primarily formulated in soap solutions to increase their invasive potential and are considered bactericidal at 5% concentration [13]. Chlorophenols do have a cloudy or milky appearance when added to water, and this factor may encourage users

to over dilute it and thus expose the bacteria to a lesser dose leading to the development of resistance [5].

The isolate from Katsina (KT 01 13) had the highest percentage growth and it was least susceptible at a lesser exposure time. Thus, there is a need to advocate for prolong exposure period during disinfection with sodium hypochlorite. A previous study reported sodium hypochlorite to have rapid bactericidal and sporicidal activity only at a high concentration which can be corrosive and not safe to use. However, at low concentrations, the vegetative bacteria can be inhibited. Sodium hypochlorite has been reported to be sensitive to light and certain materials which can easily and rapidly render the disinfectant property inactive [14].

Overall, the *E. coli* isolates were most susceptible to orthobenzyl chlorophenol-based disinfectant (Izal®) and least susceptible to chlorophenol-based disinfectant (Virkon-S®). A previous study by Okore et al. [18] showed Dettol®, Z-Germicide®, and Jik® being more effective compared to Izal® on some bacteria isolates (including *E. coli*). Another study by Agunwamba et al. [1] showed Izal® to be more effective in aerobic sewage degradation (which includes its effect on faecal coliforms) compared to Dettol®. It could mean that the susceptibility of *E. coli* to disinfection differs between strains. There is also a possibility of disinfectant resistance and biofilm formation capacity of *E. coli* in live bird markets [26]. It could also be due to the presence of the qac resistance gene and antibiotic resistance gene in *E. coli* which are linked to their resistance to disinfectants [11].

CONCLUSIONS

In conclusion, only 17% of the Live Bird Markets sampled had and used disinfectants (17% prevalence). From the LBMs that had disinfectants, the dilution in use was sufficient to kill/inhibit the growth of *Escherichia coli*, *Salmonella Enteritidis*, and *Staphylococcus aureus* at minimal exposure time, but a lower concentration will not be active against *Staphylococcus aureus*. *Escherichia coli* was isolated from 6 LBMs sampled. *E. coli* was not isolated from any LBM that used disinfectant samples except one. One particular isolate was most susceptible to the disinfectants used. The results of the susceptibility test showed that the isolates were least susceptible to chlorophenol-based disinfectant and most susceptible to orthobenzyl chlorophenol-based disinfectant.

It is therefore recommended that live bird marketers should be more proactive towards disinfection practices by ensuring that they disinfect their premises, cleaning before disinfection, and properly use disinfectants. Also, there should be a routine evaluation of the efficacy of disinfectants by the relevant authorities and encourage further research in LBMs to understand the dynamics of diseases in the LBMs.

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