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Full Length Research Paper

Antibacterial activity and phytochemical investigation of leaf and root extracts of *Aloe gilbertii Reynolds*

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The objective of the study was to test the antibacterial activities of crude extracts of roots and leaves of *Aloe gilbertii* Reynolds against clinical pathogens. The crude extracts were prepared via maceration technique employing n-hexane, acetone, chloroform, dichloromethane:methanol (50:50% V/V) and methanol solvent system. The phytochemical screening tests of the dichloromethane:methanol (50:50% V/V) root extract of *A. gilbertii* revealed the presence of alkaloids, glycosides, phenols, flavonoids, anthraquinones and terpenoids. In the same way, the phytochemical tests of dichloromethane:methanol (50:50% V/V) leaf extract of the same plant revealed the presence of alkaloids, saponins, phenols, flavonoids, anthraquinones and steroids. Antibacterial activities of both plant parts were tested against four bacterial strains namely *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Escherichia coli* using the agar well diffusion method. Root extracts were found to possess better growth inhibitory activities against all the bacterial species. The zones of inhibition were in the ranges of 8 to 23 and 8 to 18 mm, for the root and leaf extracts, respectively. The finding of the study justifies the use of the *A. gilbertii* Reynolds in traditional medicine for the treatment of various human illnesses caused by bacterial organisms; however, further investigations are needed.

Key words: Antibacterial activity, phytochemical screening, *Aloe gilbertii*, leaf extract, root extract.

INTRODUCTION

Plants have not only nutritional values but also medicinal and ritual/magical values. Literature reports revealed that medicinal plants have a long history of use in most communities in developing countries (Sofowora et al., 2013). The vast majority (70-80%) of people in the developing world consult Traditional Medical Practitioners (TMPs) for their healthcare systems (Tugume and Nyakoojo, 2019; Tezera et al., 2020). The medicinal plants contain vitamins, minerals and a variety of secondary metabolites and have been used for a long time by TMPs for the treatment of numerous human and animal diseases in various parts of the developing world (Tugume and Nyakoojo, 2019). It is believed that medicinal plants have been used and are still in use as the primary source of medicine. *Aloe gilbertii* is widely used locally by different communities in Ethiopia as a medicinal plant in the

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Figure 1. *Aloe gilbertii* species.

treatments of malaria and wound healing (Chitme et al., 2004). Aloe is a plant species with a long ethnobotanical and medicinal history around the world. The genus is comprised of approximately 420 species with centres of diversity in Southern and East Africa, the Arabian Peninsula and Madagascar. It is also reported to be an important source of biologically active compounds with well over 130 phytoconstituents isolated from the group (Wollela, 2018; Dagne et al., 2000). Aloe species are well known for their effectiveness in treating stomach ailments, gastrointestinal problems, skin diseases and constipation (Radha and Laxmipriya, 2015). They have anti-inflammatory, antiulcer and antidiabetic effects and wound healing properties (Belayneh et al., 2020; Singh et al., 2010). They are also used widely in the preparation of skincare, cosmetics products and as nutraceuticals (Upadhyay, 2018). *Aloe gilbertii* (Figure 1) is one of the *Aloes* that is endemic to Ethiopia (Sebsebe and Nordal, 2010; Wollela, 2018). Its different parts are used for the treatment of various diseases in traditional or folk remedies throughout the world (Belayneh et al., 2020). For instance, leaves and root parts have been used by local people mainly for the treatment of malaria and wounds (Fikre, 2013). Some of the pharmaceutical reports revealed that dichloromethane: methanol (50:50% V/V) root extract of *A. gilbertii* showed the presence of secondary metabolites such as flavonoids, anthraquinones, alkaloids, saponins and phenol (Mudin et al., 2018; Yadeta, 2019a). Despite its wide medicinal use and phytochemical studies, there are no as such published reports on the investigation of the antibacterial activities of root extracts of Ethiopian endemic Aloe species. This fact has initiated the present study to study the in *vitro* antibacterial activities of the root and leaf extracts of *A. gilbertii* against selected multidrug-resistant gram-positive and gram-negative bacterial pathogens.

MATERIALS AND METHODS

Plant material collection and authentication

The plant materials (root and leaf parts) were collected from July 2015 to September 2016 from Alamura Hill, on the road to Dilla and Alaba Mountain slopes of Southern Nation Nationalities People Region (SNNPR), Southern Ethiopia. The plant material was authenticated by Professor Fikre Dessalegn, Department of Botany, Addis Ababa University and the plant specimen was deposited at the Herbarium Faculty of Science, Addis Ababa University. The plant materials were dried, powdered and made ready for extraction.

Preparation of plant extract

Powdered plant materials (500 g root and 500 g leaf) were sequentially extracted with n-hexane, acetone, chloroform (2 l each) for 24 h and dichloromethane: methanol (50:50% V/V) and methanol (2 l each) for 72 h, respectively by maceration. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator at a temperature of 40°C. The resulting crude extracts of n-hexane, acetone, chloroform and methanol (100%) were discarded as the percent yields were too small to be used for the evaluation of antibacterial activities. The dichloromethane: methanol (50:50% V/V) was weighed and stored in a refrigerator until used for antimicrobial activity and phytochemical screening tests.

Phytochemical screening tests

Phytochemical screening was carried out on the crude extract of dichloromethane: methanol (50:50% V/V) roots and leaves to identify secondary metabolites. The screening was done following standard procedures reported in the literature. Test for alkaloids (Dragendroff's test): About 0.3 g of each of the crude extracts was mixed with concentrated hydrochloric acid (2 ml). The mixture was then filtered and mixed with a small amount of amyl alcohol at room temperature. Few drops of Dragendroff's reagent (Solution of potassium bismuth iodide) were added to the acid layer and a reddish-brown precipitate was observed (Ganjewala and Dipita, 2009). Test for tannins (Gelatin Test): Small amount of the extract was mixed with water and heated in a water bath. Then, a gelatin solution (0.5 ml) that contains sodium

chloride was added to the above mixture. The formation of a white precipitate indicates the presence of tannins (Saklani et al., 2012). Test for phenols: The extract (0.5 g) was dissolved in distilled water (5 ml). Then few drops of neutral 5% ferric chloride solution were added to the mixture. The formation of a dark green color was used as an indicator for the presence of phenolic compounds (Rohit, 2015).

Test for anthraquinones: About 0.5 g of the methanol extract was boiled with concentrated hydrochloric acid for a few minutes in a water bath and filtered. The filtrate was allowed to cool and an equal volume of chloroform was added to it. A Few drops of ammonia were added to the mixture and heated in a water bath. The formation of rose-pink color was inspected for the presence of arthroquinones (Evans, 2002).

Test for saponins (Froth Test): About 0.1 g of the crude extract was dissolved in water (20 ml) shaken in a graduated cylinder for 15 min. The formation of a 1 cm layer of foam indicates the presence of saponins (Roopashree et al., 2008).

Test for terpenes: About 0.25 g of extract was mixed with chloroform (2 ml) and concentrated sulfuric acid (30 ml) was added carefully to form a layer. The reddish-brown coloration of the interface was inspected for the presence of terpenes (Alamzeb et al., 2013).

Test for flavonoids (Alkaline Reagent Test): Few drops of sodium hydroxide solution were added to the extract and the formation of intense yellow color, which becomes colorless on the addition of dilute acid, indicates the presence of flavonoids (Saklani et al., 2012).

Tests for steroids (Liebermann-Burchard test): Small amount (0.1 g) of each extract was shaken with chloroform in a test tube; a few drops of acetic anhydride were added to the test tube and boiled in a water bath and rapidly cooled in iced water. Concentrated sulfuric acid (2 ml) was added to the above mixture. Formation of a brown ring at the junction of two layers and turning the upper layer to green shows the presence of steroids (Joshi et al., 2013).

Bacterial activity tests

Test organisms: The selected bacterial strains two gram-positive (*Staphylococcus aureus* (ATCC 25923), *Enterococcus faecali* (ATCC29212) and two gram negative bacteria, *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae,* (ATCC700603) were obtained and confirmed at Chromopark Research Laboratory, Trichy Road, Nammakal 637001, Tamil Nadu, India. They were maintained on Mueller-Hinton Agar medium. Twenty-four-hour-old pure cultures were prepared for use each time. All the antibacterial activity tests were carried out at Chromopark Research Laboratory, Tamil Nadu, India. The bacterial strains were reactivated by sub culturing on a nutrient broth at 37°C and maintained on a nutrient agar slant at 4°C for further activity.

In vitro antibacterial activity

Agar well diffusion assay

The antibacterial activity was carried out using the agar diffusion method (Ba-Hamdan et al., 2014). The 20 ml of Muller Hinton agar media was placed in the Petri dishes (100 mm diameter) and then the medium surface was impregnated with the 24 h grown selected bacterial strains (1.5×10^6 cells per ml). Different concentrations (5-12.5 mg) of the dichloromethane: methanol (50:50% V/V) root and leaf parts of *A. gilbertii* extracts were dispensed in separate well with the help of a micropipette incubated at 37 °C for 24 h. The dissolution of the organic extracts was facilitated with the addition of 10% (v/v) dimethyl sulfoxide (DMSO) which did not affect the growth of microorganisms (as shown by our control experiments). The formation of an inhibition zone was measured using the ruler that measured in millimeters. Lack of bacterial growth represented the antibacterial effect in the medium. The 10 µg of standard antibiotic (Ampicillin) was used as a reference drug.

Determination of minimum inhibition concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of root and leaf parts of *Aloe gilbertii* extracts showed significant antibacterial activity against gram-positive bacterial strains *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecali* (ATCC29212) and two negative bacteria, *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae,* (ATCC700603) were determined using Mueller–Hinton broth microdilution method of the Clinical and Laboratory Standards Institute, M07-A8 AS. The original stock solutions of selected plant extracts were prepared with at 50 mg extract/ml 10% DMSO solution. The MIC of tested plant extract was determined as the lowest concentration inhibiting the visual growth of the tested bacterial cultures. The initial test concentration was serially diluted in a 96 well plate to obtain final concentrations of 10, 9, 8, 7, 6 and 5mg/ml with the DMSO solution and inoculated with 5 µl of suspension containing 10^8 CFU ml^{-1} of selected bacterial strains. The 96 well plates were incubated for 24 h at 37 °C for bacterial growth. The culture intensity of each well was read at 600 nm and compared with the untreated control. The experiments were conducted in triplicates

RESULTS AND DISCUSSION

Phytochemical screening tests of *A. gilbertii* extracts

The results from the phytochemical screening of the dichloromethane: methanol (50:50% V/V) A*. gilbertii* roots extract revealed the presence of secondary metabolites such as alkaloids, glycosides, phenols, flavonoids, anthraquinones and terpenoids whereas alkaloids, saponins, phenols, flavonoids, anthraquinones and steroids were detected the leaf extract (Table 1). The present finding is consistent with previous reports by (Mudin et al. 2018) and Yadeta (2019b). In this study, glycosides and terpenes were found in the root extracts whereas these two classes of compounds were not detected from the leaves of *A. gilbertii*. Moreover, saponins and steroids were detected in leaf extracts but not in the root extract (Table 1). The presence of these secondary metabolites could be responsible for the wide medicinal uses of *A. gilbertii*.

Antibacterial activities of the crude extracts

It is known that phytochemical constituents or secondary metabolites are responsible for most of the biological activities such as antibacterial activities of medicinal plants (Anushia et al., 2009). In order to evaluate their antibacterial activities and their potential as sources of new antibacterial agents, both the root and leaf extracts were subjected to *in vitro* activity tests against four bacterial strains namely *S. aureus, E. faecalis, K. pneumoniae* and *E. coli,* using four different concentrations (5, 7.5, 10 and 12.5 mg/ml). The results indicated that the gram negative

Table 1. Secondary metabolites identified in the *A. gilbertii* roots and leaves crude extracts.

Phytoconstituents	Root extract	Leaf extract
Alkaloids	+	+
Glycosides	+	-
Saponins	-	+
Phenols	+	+
Tannins	+	+
Flavanoids	+	+
Anthraquinones	+	+
Terpenes	+	-
Steroids	-	+

Present (+); Absent (-).

Table 2. The zones of inhibitions of the root and leaf extracts of *A. gilbertii* at different concentrations.

Bacterial strain	Conc. of extracts (mg/ml)/zone of inhibition (in mm)								Zone of inhibition of Ampicillin (10 µg/ml)
	5		7.5		10		12.5		
	R.E	L.E	R.E	L.E	R.E	L.E	R.E	L.E	
E. coli	12	10	16	12	18	15	21	18	-
K. pneumoniae	-	-	8	-	11	8	14	11	-
E. faecalis	13	8	16	12	18	15	23	17	-
S. aureus	-	9	-	12	-	15	8	17	8

No zone of inhibition or no growth inhibition; R.E: root extract; L.E: leaf extract.

bacterial strains such as *K. pneumonia* at 5 mg/ml, gram positive *S. aureus* at 5, 7.5 and 10 mg/ml concentrations were found not to be sensitive to the root extract of *A. gilbertii* whereas gram positive *E. faecalis* and gram-negative *E. coli* were found to be sensitive to the extracts at 5 mg/ml concentration. At 5, 7.5, 10 and 12.5 mg/ml concentrations, the observed inhibition zones for E. *faecalis* and *E. coli* were 13, 16, 18, 23 and 12, 16, 18, 21 mm, respectively (Table 2). The observed inhibition zone for the root extract at a concentration of 12.5 mg/ml was found to be the same (8 mm) as that of the reference antibiotic drug (Ampicillin -10 µg/ml) against *S. aureus* (Table 2). The observed inhibitions zones were also found relatively to be significant at higher concentrations than that of the reference drug for most of the bacterial strains (*E.coli, K. pneumoniae and E. faecalis*). The data also showed that the zones of inhibition increase with the increasing concentrations of the extracts (Table 2). A similar trend was observed for the leaf extracts on three bacterial strains (*E. coli, E. faecalis* and *S. aureus*). On the other hand, no growth of *K. pneumoniae* was observed at low concentrations (5 and 7.5 mg/ml) (Table 2). Despite their comparable inhibition zones (at 12.5 mg/ml), the root extract was found to be effective against the four bacterial strains whereas the leaf extracts were more effective than root extract only against one bacterial species namely, *S. aureus*. The difference in inhibition activities of root and leaf extracts could be attributed to the absence of some phytochemicals (such as, terpenes and glycosides) in the leaf extract (Table 1). This is consistent with the literature reports that discuss antibacterial activities of terpenes (Yoshihiro et al., 2008; Naoko et al., 2008), alkaloids (Singh and Verma, 2011) and polyphenols (Dua et al., 2013) as well as glycosides (Nazemiyeh et al., 2008; Sameerah et al., 2013). The fact that the root extract is active against both gram-negative (*K. pneumoniae* and *E. coli*) and gram-positive (*E. faecalis* and *S. aureus*) bacteria suggests the possibility of developing new broad-spectrum agents that could be used for the treatment of bacterial infections that are resistant to currently existing drugs.

The minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the root and leaf extracts of *A. gilbertii* were assessed by 96 well plate broth micro dilution methods with a concentration range from 5mg /ml to 10 mg /ml. Only the tested bacteria, which were highly susceptible to the selected plant extracts, were taken for determining the MIC. The maximal zones of inhibition and MIC values for tested bacterial strains, which were sensitive to the *A. gilbertii* root and leaf extracts, were in the range of 8-23 mm and MIC values of 1 - 8.5 mg/ml. As shown in Table 3, among the tested plant

Table 3. Minimum inhibitory concentration (MIC) of extracts on selected bacterial strains.

Plant extract	*S. aureus* (ATCC25923) (mg/ml)	*E. feacalis (*ATCC 29212) (mg/ml)	*E. coli* (ATCC25922) (mg/ml)	*K. Pneumonia* (ATCC 700603) (mg/ml)
Leaf	1	1	1	6
Root	8.5	1	1	3.5

extracts, a root extract of *A. gilbertii* showed strong antibacterial activity against *E. faecalis* and *E. coli* with a significant zone of inhibition were in the range of 13-23 mm and 12-21 mm respectively with MIC values of 1 mg /ml.

Conclusion

This work is one of the few attempts to analyze the phytochemical constituents and *in vitro* antibacterial activity of polar extracts of the roots and leaf of *A. gilbertii* indigenous to Ethiopian flora. Phytochemical screening tests of the crude dichloromethane:methanol (50:50% V/V) root extract of *A. gilbertii* revealed the presence of alkaloids, terpenoids, anthraquinones, phenols, flavonoids, steroids, glycosides, saponins whereas alkaloids, anthraquinones, phenols, flavonoids, steroids and saponins were detected in the leaf extracts. In agreement with the previous study, the wide traditional use of the plant may be attributed to its rich anthraquinones and phenolic compound constituents. Moreover, the *in vitro* antibacterial activity of root and leaf extracts of *A. gilbertii* showed significant antibacterial activity. Thus, further work is recommended on this endemic plant to validate its use in traditional use and to identify more bioactive secondary metabolites and compounds in support of its traditional use.

CONFLICT OF INTEREST

The authors have not declared any conflict of interest.

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Antibiotic sensitivity patterns of nosocomial infections bacterial isolates in National Hospital in Niamey, Niger

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Nosocomial infections are recognized as a global public health issue, with causative bacteria often exhibiting high levels of antibiotic resistance. However, there is a paucity of data on multidrug-resistant bacteria in nosocomial infections in Niger. This study aimed to determine the antibiotic sensitivity of bacteria isolated from specimens of patients suffering from nosocomial infections. Samples collected from patients meeting the case definition for nosocomial infections, including pus, blood, and urine, were cultured in the hospital's microbiology laboratory following established standard operating procedures. Bacterial isolation and identification were carried out before conducting antibiotic sensitivity testing using the BioMérieux VITEK®2 method. A total of 386 isolates were examined, with *Escherichia coli* representing 61.4%, *Klebsiella species* 23.8%, and Proteus mirabilis only 0.25%. E. coli exhibited a high level of resistance reaching 96.20% to ceftriaxone, but susceptible to 92.83% to meropenem. Klebsiella species also showed high resistance level to antibiotics, including 90% to piperacillin but susceptible to 95.66% to meropenem. *Staphylococcus aureus* demonstrated 82.95% resistance to ciprofloxacin but remained susceptible to fusidic acid (82.64%). Notably, the major pathogens also produced extended-spectrum beta-lactamase at rates of 21.94, 44.44 and 42.39% for *E. coli, Enterobacter, and Klebsiella*, respectively. The antibiotic sensitivity testing revealed resistance and co-resistance to many antibiotics, posing significant therapeutic challenges and necessitating the use of carbapenems, considered the last line of therapy against some multidrug-resistant bacteria in the hospital. Therefore, there is a critical need to reactivate the nosocomial infection control committee, and the implementation of antimicrobial surveillance practices is highly recommended.

Key words: Nosocomial infections, antibiotic susceptibility pattern, bacterial isolates, Niamey, Niger.

INTRODUCTION

Health care-associated infections (HAIs), formerly known as nosocomial infections, refer to infections acquired during the course of receiving healthcare that were not present at the time of admission. These infections can occur in various healthcare settings, including hospitals, long-term care facilities, and ambulatory settings, and

may even manifest after discharge. HAIs also encompass occupational infections that can affect healthcare staff (WHO, 2002).

Healthcare-associated infections (HAIs) contribute significantly to morbidity, mortality, and financial burdens on patients, families, and healthcare systems. Studies have indicated that nosocomial infections occur in 5 to 10% of all hospitalizations in Europe and North America, and in more than 40% of hospitalizations in parts of Asia, Latin America, and Sub-Saharan Africa (WHO, 2002), although the overall burden of nosocomial infections in Sub-Saharan Africa has been described to range from 2.5 to 14% (Nejad et al., 2011). A systematic review of the literature has revealed a highly fragmented picture of the endemic burden of HAIs in the developing world (WHO, 2011).

The bacteria commonly responsible for nosocomial infections include *Staphylococcus aureus, Streptococcus spp, Bacillus cereus, Acinetobacter species, coagulase-negative staphylococci, Enterococci, Pseudomonas aeruginosa, Legionella,* and members of the Enterobacteriaceae family such as *E. coli, Proteus mirabilis, Salmonella species, Serratia marcescens, and Klebsiella pneumoniae.* However, the most frequently reported nosocomial pathogens have been *E. coli, S. aureus, Enterococci, and P. aeruginosa* (Horan et al., 2008).

The discovery of antibiotics has been a relief for humanity because these remedies have significantly reduced the incidence of infectious diseases, especially in developing countries (Guessennd et al., 2008). However, the emergence of multi-drug resistant organisms poses another complication associated with healthcare-associated infections (HAIs).

HAIs affect 3.2% of all hospitalized patients in the United States, 6.5% in the European Union/European Economic Area, and the worldwide prevalence is likely much higher (Magill et al., 2018; Suetens et al., 2018; Allegranzi et al., 2011). The global burden of HAIs was initially unknown due to the absence of surveillance systems for HAIs. However, there has been a significant effort by infection prevention and control programs to develop surveillance systems and implement infection control methods (Storr et al., 2017).

A high prevalence of nosocomial infections attributable to multidrug-resistant bacterial strains has been reported in countries worldwide (Seligman et al., 2013; Costa et al., 2015).

Western sub-Saharan Africa reported the highest burden, with 27.3 deaths per 100,000 (20.9 to 35.3) attributable to antimicrobial resistance (AMR) and 114.8 deaths per 100,000 (90.4 to 145.3) associated with AMR. All four regions of Sub-Saharan Africa and South Asia had all-age death rates associated with bacterial AMR higher than 75 per 100,000.

Although Sub-Saharan Africa had the highest all-age death rate attributable to and associated with AMR, the percentage of all infectious deaths attributable to AMR was lower in this super-region, as indicated in a systematic analysis concerning the Global Burden of Bacterial Antimicrobial Resistance (2019).

In Niger, few research studies have been conducted on healthcare-associated infections (HAI), and data concerning the antimicrobial resistance of incriminated bacterial isolates are very scanty. In the present study, we evaluate the prevalence, distribution, and antimicrobial susceptibility rates of bacterial strains in patients with HAIs over a 2-year period to enhance healthcare practices and support probabilistic antibiotic prescription in the hospital.

MATERIALS AND METHODS

Study design and site

The study was conducted at the National Hospital of Niamey, which has a capacity of 1000 beds, spanning from January 2021 to June 2022. Biological samples, including urine, blood, and pus, were collected from patients diagnosed with Healthcare-Associated Infections (HAI) based on the specified case definition. These samples underwent analysis in the hospital's bacteriology laboratory for the isolation, identification, and antibiotic susceptibility testing of pathogenic bacterial isolates.

Culture and identification of bacterial isolates

Bacterial strains were isolated from the culture of various biological specimens. Urine samples were inoculated onto CLED (cystine lactose electrolyte deficient) agar plates and incubated at 37ºC for 18 to 24 h. Pus samples were inoculated onto nutrient agar plates, sheep blood agar, MacConkey, and Mannitol salt agar plates. Nutrient agar, MacConkey, and Mannitol salt agar plates were incubated at 37ºC for 18 to 24 h in aerobic conditions, while sheep blood agar plates were incubated under 5 to 10% CO2 for 18 to 24 h. Blood samples were inoculated and incubated in culture bottles with Bact/Alert 3D 60 Biomérieux at 37ºC. The bottles were incubated to detect growth. When no bacterial growth was observed, the culture was declared negative. However, for positive cultures, the morphologies of the detected bacteria were described, and Gram staining was performed before biochemical tests (oxidase and catalase tests).

The final identification of the microorganisms was accomplished using the Biomérieux VITEK® 2 method. The identified isolates were preserved at -80ºC for further analysis.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of the isolates was conducted using Biomérieux VITEK®2 method. Only fresh cultures of the

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pathogenic microorganisms were used for antibiotic sensitivity test. The results were obtained after 24 h incubation. The choice of cards used depended on recommendations of the above-mentioned method.

Detection of extended-spectrum beta-lactamase (ESBL) by DDST

Detection of ESBL was performed following the recommendations of EUCAST-CASFM (2021). The detection of ESBL was conducted only on isolates of *E. coli, Klebsiella, and Enterobacter* species (Table 8). A Mueller-Hinton agar (MHA) plate was inoculated with a suspension made from an overnight nutrient agar culture of the test strain, as recommended for a standard disk diffusion susceptibility test. Disks containing the standard 30µg of ceftazidim, ceftriaxone, aztreonam, and 10 µg of cefpodoxime were placed 15 mm apart (edge to edge), and an amoxicillin + clavulanic acid disk containing 10 µg of the latter compound was placed in the center of the plate. The disk edge-to-edge distance recommended here was reported to have greater sensitivity than the previous distance of 20 to 30 mm. Following incubation for 16 to 20 h at 35ºC, any enhancement of the zone of inhibition between a beta-lactam disk and that containing the beta-lactamase inhibitor indicated the presence of an extended-spectrum beta-lactamase.

Phenotypic detection of methicillin resistant Staphylococcus aureus (MRSA)

Detection of MRSA strains was conducted using cefoxitin as recommended by EUCAST-CASFM (2021). All the isolates were subjected to cefoxitin disc diffusion test using a 30µg disc. A 0.5 Mc Farland standard suspension of the isolate was made and lawn culture done on MHA plate. Plates were incubated at 37ºC for 18 h and inhibition zone diameters were measured. An inhibition zone diameter r≤ 22mm was reported as oxacillin resistant and ≥ 22 mm was considered as oxacillin sensitive.

RESULTS

Patients and samples characteristics

Patients of both sexes were affected by HAI, but males represented with 58% of cases, whereas the females do contribute to 42% in our study. Samples were collected in four departments of the hospital: urology, surgery, medicine and pediatric ward. Urines samples represented 72.02% of samples, pus and catheters for 25.64% and blood samples account for only 2.33%.

Bacterial strains and provenance

During our study, 386 bacterial strains were isolated from non-repetitive samples derived from suspected HAI patients. Seven different strains were isolated from samples mostly collected in urology department (Figure 1). *E. coli* (61.4%) was the most prevalent of the isolates followed by *Klebsiella species* (23.8%) whereas *Proteus mirabilis* accounted only for 0.25% (Figure 2).

DISCUSSION

Patients and samples characteristics

In our study, (HAIs) related to urinary tract infections were the most frequent, accounting for more than 50% of documented cases. These findings align with results reported in Congo Brazzaville (Bopaka et al., 2021). The prevalence of urinary tract infections in our study was higher than that reported in Algeria (Atifa et al., 2005), although the prevalence of surgical HAIs was almost similar. The higher prevalence of urinary tract infections may be explained by the brief stay of patients in an emergency care unit and the use of urinary catheters. The sex ratio in our study was 1.36, which was lower than that found in Tunisia (Latifa et al., 2014), where the sex ratio was 2.48. This difference may be attributed to the lower number of included patients in our survey and the existence of health reference centers for women.

The bacteria identified in our study included *E. coli, Klebsiella species, Enterobacter species, Staphylococcus aureus, Acinetobacter, Pseudomonas, and Proteus mirabilis.* These pathogens have been implicated as major causes of nosocomial infections in previous studies conducted in Benin Republic (Afle et al., 2018) and the Democratic Republic of Congo (Bukasa et al., 2021). These results suggest that these pathogens are prevalent in various bacterial infections in hospitals and play a significant role in causing HAIs, particularly in conditions of poor hygiene.

Patterns of sensitivity to antibiotics

Sensitivity of E. coli isolates

During the study, bacterial isolates exhibited a high level of resistance to antibiotics. *E. coli* showed more than 80% resistance to ampicillin, amoxicillin + clavulanic acid, and ceftriaxone (Table 2). Similar results have been reported in Niger (Fody et al., 2015), Nigeria (Odumosu and Akintimehin, 2015), and Cameroon (Clotilde et al., 2013). The pathogen also demonstrated co-resistance to ciprofloxacin (85.23%). High levels of resistance of E. coli to ciprofloxacin have been reported in many studies in Niger (Fody et al., 2015), Cameroon (Clotilde et al., 2013), and Senegal (Leye et al., 2019). These results may be explained by repeated exposures to antibiotics and the misuse of such drugs over the years.

The *E. coli* isolates used in this study also produced extended-spectrum beta-lactamase (ESBL). Most of these isolates were obtained from the urine of male patients in the age group over 60 years. This finding differs from that reported in Nigeria (Akanbi et al., 2013), where no differences were apparent in the gender distribution of ESBL-producing E*. coli*.

Our results may be attributed to the high number of

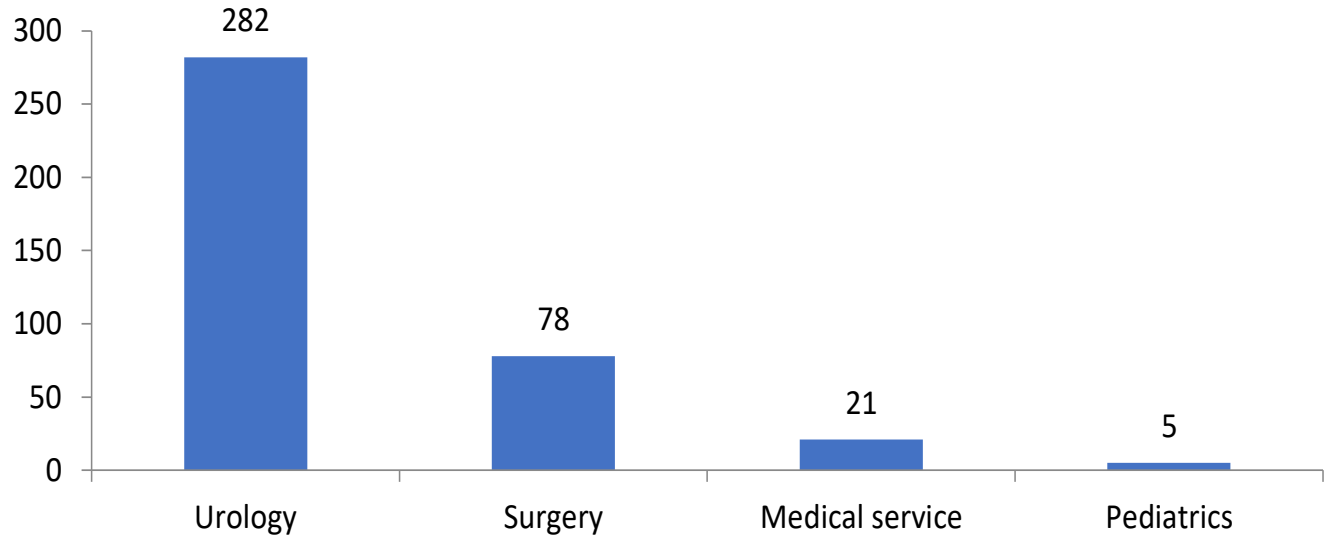

Figure 1. Sample provenance.

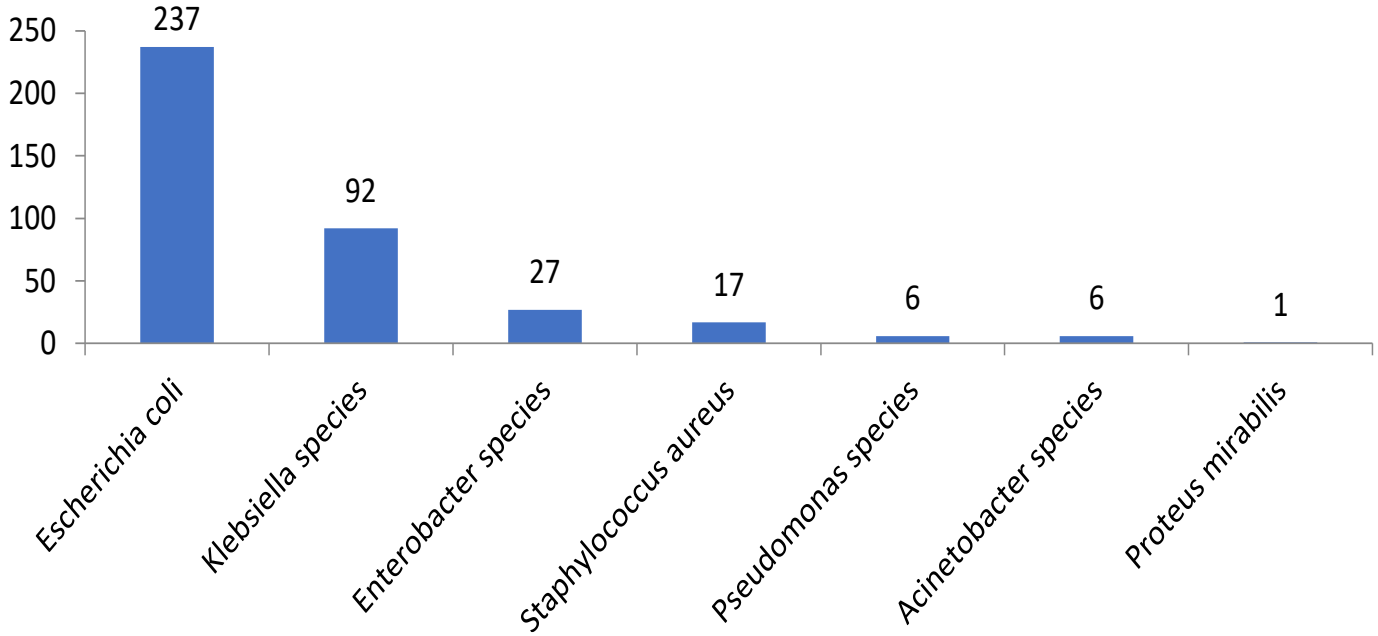

Figure 2. Prevalence of bacterial isolates.

urine samples and our sex ratio. In terms of age groups, we observed a prevalence of 35.89% of ESBL-producing E. coli in the age group of 61-80 years (Table 1). This result differs from that reported in a study conducted in Nigeria (Akanbi et al., 2013), which suggested that there was no relationship between ESBL-producing E. coli and age. This difference may be justified by the fact that patients in this age group may likely develop urinary infections due to a weakened immune system and prolonged exposure to antibiotics.

Sensitivity of Klebsiella species

Another pathogen isolated from nosocomial infections was *Klebsiella* species. The pathogen also showed a high level of resistance: 89.13% to amoxicillin + clavulanic acid, 92.39% to ceftriaxone, 67.89% to ciprofloxacin, and 4.5% to imipenem (Table 3). Additionally, 42.33% of *Klebsiella* isolates produced ESBL, most commonly in the age group of 61-80 years. High resistance levels of the microorganism have been

Table 1. Bacterial isolates according to age groups.

Bacterial isolate	Age group (year-old)			
	0-20	21-40	41-60	>60
Escherichia coli	33	49	76	79
Klebsiella species	20	11	26	35
Enterobacter species	5	9	4	9
Pseudomonas species	0	4	0	2
Acinetobacter species	0	1	3	2
Staphylococcus aureus	6	5	6	0
Proteus mirabilis	0	1	0	0

Table 2. Patterns of *Escherichia coli* sensitivity to antibiotics.

Antibiotic	Number resistance (%)	Number susceptible (%)
Ampicillin	235(99.15)	2(0 .85)
Amoxicillin	220(92.83)	17(7.17)
Amoxicillin + clavulanic acid	195(82.27)	42(17.73)
Gentamycin	193(81.43)	44(18.57)
Ticacillin	230(97.04)	7(2.96)
Ticarcillin +clavulanicacid	202(85.23)	35(14.77)
Piperacilin+tazobactam	192(81.01)	45(18.99)
Ceftriaxon	228(96.20)	9(3.8)
Cefotaxim	228(96.20)	9(3.8)
Imipenem	17(7.17)	220(92.83)
Meropenem	17(7.17)	220(92.83)
Kanamycin	159(67.08)	78(32.92)
Amikacyne	146(61.60)	91(38.4)
Ciprofloxacin	202(85.23)	35(14.77)
Fosfomycin	89(50.29)	88(49.71)
Nitrofurantoin	92(51.98)	85(48.02)

reported in Senegal (Leye et al., 2019).

These results are also similar to those reported in Iran (Moini et al., 2015), where 29.6% of multidrug-resistant *Klebsiella* species were isolated from samples of patients aged 60 and older. Furthermore, most ESBL-producing *Klebsiella* species were isolated from the urine of male patients. This result is similar to that found in Morocco (Sbiti et al., 2017) and may be dependent on the sex ratio and sampling. This can be explained by the fact that patients suffering from urinary tract infections in our study were mostly elderly and had long-lasting exposure to antibiotics.

Sensitivity of Enterobacter species

Enterobacter showed resistance to antibiotics: 88.88% to ciprofloxacin, 85.18% to ceftriaxone, and 44.44% of *Enterobacter* species were ESBL positive, mostly isolated from male patients and pus specimens in the age group of 0–20 years old. These results are similar to those found in Togo (Toudji et al., 2017), where a high resistance and co-resistance of the microorganism have been noted. Although *Enterobacter* species showed a high level of resistance to some antibiotics, it nevertheless remained susceptible to *imipenem* (88.88%) (Table 4), providing a chance for infected patients by the microorganism to be successfully treated with this drug. The susceptibility of this pathogen to imipenem could be explained by the fact that, until recently, the drug was not prescribed abusively to patients in our hospital.

In our study, 44.44% of *Enterobacter* species produced ESBL. Regarding ESBL-producing isolates, a similar result has been reported in Nepal (Jeny and Nabaraj, 2015), where most ESBL-producing *Enterobacteriaceae* were isolated from pus samples. Such a rate of resistance to beta-lactams may likely be explained by the fact that these antibiotics were the most prescribed in the hospital, indicating that earlier colonization by ESBL-producing isolates can occur in the hospital in nosocomial

Table 3. Patterns of *Klebsiella species* sensitivity to antibiotics.

Antibiotic	Number Resistance (%)	Number Susceptible (%)
Piperacillin	83(90)	9(10)
Amoxicillin+clavulanicacid	82(89.13)	10(10.87)
Gentamicin	64(69.56)	28(30.44)
cefoxitin	56(60.86)	36(39.14)
Piperacillin+tazobactam	80(77.17)	12(22.83)
Ceftriaxon	85(92.39)	7(7.61)
Imipenem	4(4.34)	88(95.66)
cefotaxim	85(92.39)	7(7.61)
Meropenem	4(4.34)	88(95.66)
Amikacyne	45(48.91)	47(51.09)
Ciprofloxacin	62(67.39)	30(32.39)
Fosfomycin	42(56.75)	32(43.25)
Nitrufirantoine	66(89.19)	8(10.81)
ticarcilline	79(85.86)	13(14.14)
Ticarcillin + clavulanic acid	73(79.34)	19(20.66)
Kanamycine	71(77.17)	21(20.83)

Table 4. Patterns of *Enterobacter species* sensitivity to antibiotics.

Antibiotic	Number Resistance (%)	Number Susceptible (%)
Piperacillin	17(62.96)	10(37.04)
Piperacillin + tazobactam	16(59.25)	11(40.75)
Kanamycin	18(66.66)	11(34.34)
Gentamycin	17(62.96)	10(37.04)
Amikacyn	11(40.74)	16(59.26)
cefoxitin	16(59.25)	11(40.75)
Cefotaxim	23(85.18)	4(14.82)
Ticarcillin	25(92.59)	2(7 .41)
Ticarcillin+ clavulanic acid	22(81.48)	5(18.52)
Céftriaxon	23(85.18)	4(14.82)
Imipenem	3(11.11)	24(88.89)
Meropenem	3(11.11)	24(88.89)
Ciprofloxacin	24(88.89)	3(11.11)

infections, as reported in Tanzania (Mshana et al., 2016).

Sensitivity of Pseudomonas species

Pseudomonas was also isolated in our samples during our survey. Many studies conducted in Tunisia (Fki et al., 2018) and in Benin Republic (Afle et al., 2018) have reported the microorganism as one of the pathogenic organisms in HAI. The rate of resistance to some antibiotics (50% to ciprofloxacin and 50% to ceftazidim) was almost similar to that reported in Tunisia (Krir et al., 2019), although the susceptibility of the pathogen to imipenem was quite different (Table 5). The study duration and the number of isolates can explain such results

Sensitivity of Acinetobacter species

During our survey, *Acinetobacter* species exhibited high resistance to some antibiotics to the extent of 100% to ticarcillin, 83.33% to piperacillin, 66.66% to ciprofloxacin, and 50% to ceftazidim (Table 6). Such a level of resistance has been noted in Senegal (Leye et al., 2019), where a higher resistance level to these drugs was found. The abuse prescription of drugs and also poor hygiene conditions in the hospital are factors behind such a situation.

Sensitivity of staphylococcus aureus

During our study, *S. aureus* represented only 4.4% of

Table 5. Patterns of *Pseudomonas species* sensitivity to antibiotics.

Antibiotics	Number resistant (%)	Number susceptible (%)
Piperacillin	4(66.66)	2(33.34)
Piperacillin+ tazobactam	4(66.66)	2(33.34)
Gentamycin	4(66.66)	2(33.34)
Amikacyn	2(33.34)	4(66.66)
Ceftazidim	3(50)	3(50)
Ticarcillin	4(66.66)	2(33.34)
Ticarcillin+ clavulanic acid	3(50)	3(50)
Imipenem	2(33.34)	4(66.66)
Meropenem	2(33.34)	4(66.66)
Ciprofloxacin	3(50)	3(50)

Table 6. Pattern of *Acinetobacter species* sensitivity to antibiotics.

Antibiotic	Number resistance (%)	Number susceptible (%)
Piperacillin	5(83.33)	1(16.67)
Piperacillin+ tazobactam	3(50)	3(50)
Ticarcillin	5(83.33)	1(16.67)
Ticarcillin + clavulanic acid	4(66.66)	2(33.34)
Gentamycin	4(66.66)	2(33.34)
Amikacyn	0(0)	6(100)
Ceftazidim	3(50)	3(50)
Imipenem	2(33.34)	4(66.66)
Meropenem	2(33.34)	4(66.66)
Ciprofloxacin	4(66.66)	2(33.34)

Table 7. Pattern of ***S. aureus*** sensitivity to antibiotics.

Antibiotic	Number resistance (%)	Number susceptible (%)
Kanamycin	4(23.52)	13(76.48)
Tobramycin	3(17.64)	14(82.64)
Gentamycin	3(17.64)	14(82.64)
Erythromycin	8(47.05)	9(52.95)
Tetracyclin	9(52.94)	8(47.06)
Oxacilline	9(52.94)	8(47.06)
Ciprofloxacin	14(82.35)	3(17.65)
Fusidicacid	3(17.64)	14(82.36)

isolates and showed resistance to some antibiotics: 47.05% to erythromycin and 52.94% to tetracycline and oxacillin (Table 7), respectively. The prevalence of methicillin-resistant isolates, although comparable to that reported in Ethiopia (Tadesse et al., 2018), was different from that reported in the Democratic Republic of Congo (Lukuke et al., 2017), where a higher level of resistance to some antibiotics had been found. The low number of isolates during our study may likely explain such a situation.

Conclusion

This study revealed high resistance of bacterial isolates to commonly used antibiotics in the hospital. High susceptibility rates were observed with carbapenems, considered as the last line of treatment. Therefore, there

Table 8. Characteristics of ESBL bacterial strains.

Characteristics of ESBL bacterial strains	Bacterial strains [N(%]		
	Escherichia coli	***Enterobacter* species**	***Klebsiella* species)**
ESBL positive	52	12	39
Biological sample			
-Urines	35(67.30)	4(33.33)	31(74.48)
-Pus	17 (32.70)	8(66.66)	8(20.51)
Gender of patients			
-Male	31(59.6)	7(58.33)	33(84.61)
-Female	21(40.39)	5(41.33)	6(38.15)
Age group (y.o)			
0-20	10(25.64)	5(41.66)	13(33.33)
21-40	12(30.76)	2(16.66)	5(12.82)
41-60	14(35.89)	2(16.66)	5(12.82)
61-80	14(35.89)	2(16.66)	13(33.33)
≥ 81	2(5.12)	1(8.33)	3(7.69)

is a need to establish antimicrobial surveillance practices in the hospital to reduce the burden of healthcare-associated infections (HAI) in Niamey, Niger.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Review

Microbiological quality and safety of poultry processed in Africa: A review

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Poultry is the second most consumed meat in the world. In Africa, chicken production and processing are practiced both formally and informally, with smallholders constituting the majority in this sector. Informal practices are vulnerable to the production and processing of chicken, which is easily contaminated by pathogens such as *Escherichia coli, Staphylococcus aureus, Salmonella,* and *Campylobacter*. The growth of the poultry industry in Africa, coupled with intensive production, has led to the indiscriminate use of antibiotics and the development of antimicrobial resistance, posing a risk to the health of consumers. However, there are limited studies evaluating the quality and safety of chicken consumed in Africa. Several studies report that chicken is the main vehicle for pathogens related to foodborne diseases, suggesting that foodborne diseases pose a threat to human health. Adequate hygiene and safety practices by producers and processors are suggested as the main intervention. These practices would need to be followed by laboratory analysis and inspection to assess chicken quality and prompt changes in behavior, attitudes, and practices to reduce contamination and promote the rational use of antimicrobials. This review provides an overview of the quality and microbiological safety of processed chicken in Africa. It delves into details about the poultry sector, covering production, slaughter, and processing of chicken. The review highlights the sources and mechanisms of poultry contamination, describes diseases transmitted through the consumption of poultry, presents data on the quality and microbiological safety of chicken, proposes good practices in chicken production and processing, discusses the occurrence of antimicrobial resistance and antibiotic residues, and presents alternatives against contamination and antimicrobial resistance as potential tools for the production of healthy and safe foods.

Key words: Poultry production, microbiological quality, antimicrobial resistance, antibiotic residue.

INTRODUCTION

Poultry production is the second leading animal production sectors in the world with more than 80,086 million metric tons (MT) and Africa produces very little (2.5 million MT) (Mudenda et al., 2022). The major poultry producing countries in Africa is South Africa (1.9 million MT). The poultry sector in Nigeria is the second-

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largest in Africa (Olutumise et al., 2023). Mozambique ranks the lowest in poultry production accounting (135, 798 MT). In the developing countries, poultry production mainly (80.0%) is mainly by smallholders and is a significant source of protein and income for women across the world, especially in Africa (Wong et al., 2017; Assefa et al., 2023; Gukut et al., 2023).

In Africa, poultry production systems involve extensive, semi-intensive and intensive backyard/free range rearing and smallholders raise poultry in an artisanal way, with few work tools and knowledge, use family labor and are located in large urban centers (Birgen et al., 2020; Ramtahal et al., 2022). Smallholders' production is a significant source of food and income, with women and youth more actively involved in earlier stages of the value chain, such as poultry production and processing, particularly at the farm level.

Ariel V Garsow et al. (2022) study revealed that women and youth were the main smallholder poultry producers on the farm and, due to their direct involvement in poultry management and production may be at greater potential risk of exposure to foodborne pathogens. Most poultry produced by smallholders is processed in informal markets, where conventional methods of regulation and inspection often fail and a good deal of food products derived from livestock are potentially unsafe (Garsow et al., 2022; Malong, 2022; Ramtahal et al., 2022).

Increases in purchasing power, urbanization and advances in breeding have given rise to more productive poultry, but which need management to avoid contamination and ensure safe food (Ramtahal et al., 2022). However, poultry are considered to be the main vehicle of foodborne pathogens, such as *Campylobacter* and *Salmonella*, implicated in foodborne outbreaks and the presence of these pathogens tends to deplete their nutritional value.

Poultry foods are characterized by a higher level of bacterial contamination because they are a good substrate for the development of microorganisms, both those that cause meat deterioration and pathogenic microorganisms. Bacteria in meat can come from the chicken themselves, from habitat and can also be influenced by processing conditions (Sokolowicz et al., 2021). Food safety has become a major worldwide issue, and the majority of underdeveloped nations suffer greatly from the dangers of foodborne illness. Microbes are the primary cause of foodborne disease because they are evolving to become resistant to numerous food manufacturing and processing processes. As a result, they constitute a potential hazard to human health (Malong, 2022; Otwey and Kunadu, 2022).

In Africa, foodborne disease and microbiological contamination of food are common occurrences; however, the majority of these instances go unreported and unidentified (Amoako et al., 2020). According to Akhtar et al. (2014), Botulism, campylobacteriosis, *Escherichia coli* infection, *Staphylococcus aureus infection,* salmonellosis, listeriosis and Cholera are of particular concern from a food security perspective in the poorest economies in Africa.

The prevalence of foodborne illness still remains a cause of morbidity and death, although not well documented due to inadequate monitoring and surveillance, it contributes to economic decline in underdeveloped countries. Economic constraints, political instability, lack of infrastructure and lack of adherence to existing food laws are significant markers of an unsafe food supply. Sub-Saharan Africa suffers huge economic losses due to diarrheal diseases (30.0%), suggesting foodborne illness and food safety issues (Akhtar et al., 2014).

Compared to other continents in the world, Africa has the highest burden of foodborne disease with 100 million infections and 140,000 deaths each year. The agents of foodborne diseases are *Vibrio cholerae*, *entero pathogenic E. coli*, *Salmonella enteric, Campylobacter* spp. and *Listeria monocytogenes* (Asfaw et al., 2020; Birgen et al., 2020*)*. Therefore, monitoring of quality and safety along the poultry value chain is critical for public health. In Africa, there are few studies that evaluate the quality and microbiological safety of chicken and the results reveal that there are pathogenic that can cause diseases to consumers; other studies report the abusive use of antimicrobials and resistance to antibiotics. Many researches in the world have reported the indiscriminate use of antimicrobial agents, both for therapeutic and non-therapeutic purposes, mainly in poultry, leading to the development of antimicrobial resistance in pathogens of food origin, which represents a threat to public health for humans.

Additionally Selaledi et al. (2020) concluded that antibiotic resistance in *Campylobacter jejuni* is influenced by the use of antibiotics in healthy and diseased animals on poultry farms. However, in most African countries, laboratory analysis of food samples is not always accessible and there is a tendency to focus on formal rather than informal markets for monitoring food quality and safety (Kunadu et al., 2020; Otwey and Kunadu, 2022) . The main objective of this article is to review the Microbiological Quality and Safety of chicken produced and processed in the African continent, diseases caused by chicken consumption, suggest good practices in poultry production and processing and explore alternatives to reduce microbiological contamination in the Poultry processing chain and antibiotics in poultry production.

POULTRY PRODUCTION IN AFRICA

In Africa, poultry farming ranks second and represents 24% of total meat production. Therefore, chicken production is an important source of protein and also contributes to the generation of income and employment

for families with low-income. In most African countries, poultry productions systems involve extensive, semi-intensive and intensive backyard/free range rearing (Ramtahal et al., 2022; Sime, 2022). On farms, poultry farmers use deep litter for broilers and battery cages for laying hens. The extensive farming system is a more common practice in Africa; it guarantees food security, employment and is a source of income for many disadvantaged families (Ramtahal et al., 2022).

Poultry production is important for Africa, where about 80% of the poultry produced is raised by smallholders, it contributes to the economy, nutrition and people livelihoods but there are challenges from endemic disease outbreaks (Gukut et al., 2023). Small-scale poultry production systems, consisting mainly of chicken, represent the majority of the poultry population in Africa. Poultry meat production is based on raising young birds for slaughter (broilers). Despite the importance of Poultry production, farmers still experience economic difficulties and losses due to disease outbreaks. Therefore, the application of practices, standard health management (HMPs) is very important as these practices improve the welfare of animals and increase the production and income of farmers (Olutumise et al., 2023).

There are improvements in public health facilities but bacterial infections remain a major public health problem worldwide. Asfaw et al. (2020), point out that salmonellosis is more prevalent in areas of intensive livestock farming, mainly in chicken production. The authors add that chicken infection leads to fecal elimination in the environment, and the contamination of meat and eggs are the main chain of human infections by *Salmonella*. In addition to the contact of workers in farms and slaughterhouses and the risk of developing resistance to antibiotics in humans and animals is one of the main reasons for control in animals.

Assefa et al. (2023) report that standards in the informal food sector are non-existent or deficient with little regulatory oversight in sub-Saharan Africa. Although animal products pose a risk in terms of transmitting foodborne illnesses, they are vital components of the diets and livelihoods of the majority on the African continent. The authors add that food safety standards are poor and infrastructure is limited, to improve it is necessary to intervene in the awareness of workers through educational campaigns, accompanied by good regulation and supervision as a way to protect consumers from foodborne illnesses.

The production of chicken in extensive and intensive systems has brought dependence on the use of antibiotics to sustain poultry health and economic stability. Many studies have reported the indiscriminate use of antimicrobial agents, mainly in poultry, leading to the development of antimicrobial resistance in food-borne pathogens. Poultry production has been identified as a hot spot for the development of antimicrobial resistance and the transfer of drug-resistant microorganisms between animal feed producers and humans, due to the high and chronic use of antibiotics (Bamidele et al., 2022; Ramtahal et al., 2022). In the review, Melissa A Ramtahal et al. (2022) point out that the prevalence of *Salmonella* in extensive poultry farming systems may be underreported in Africa, as there is no routine surveillance program to monitor food safety. The authors add that the use of antibiotics in poultry has allowed the emergence of resistant bacterial strains in Africa.

The lack of adequate genomic laboratory facilities to detect resistance-conferring genes in *Salmonella* isolates across the continent contributes to this situation. In the review carried out by Ramtahal et al. (2022) it was confirmed that *Salmonella* Enteritidis, *Salmonella* Kentucky and *Salmonella* Typhimurium are widely distributed throughout the African continent and have also emerged as antibiotic-resistant and multi-resistant strains in the poultry industry. Serotyping is not routinely performed in Africa and the lack of strict hygienic practices prevent the containment of *Salmonella*, which can cause contamination of poultry and eggs along the processing line, from farm, scalding, plucking, evisceration and final product.

The spread of antibiotic-resistant bacteria from farm animals to humans and the environment is a major global health concern (Mudenda et al., 2022; Serwecińska, 2020). According to Bamidele et al. (2022), sulfonamides rank third on the list of commonly used antibiotics in small poultry production systems in Nigeria. The indiscriminate use of antibiotics, both therapeutic and non-therapeutic, in poultry production systems poses a public health threat to humans because it requires treatment with third-line medication and takes time to recover. Therefore, the use of probiotics and prebiotics in poultry production can be an alternative.

POULTRY SLAUGHTERING AND PROCESSING IN AFRICA

In underdeveloped and low-income countries, chicken is a more accessible source of protein, a source of employment and income for many families. Poultry refers to domesticated chicken such as indigenous and commercial chickens, turkeys, ducks, geese, quails, pigeons and guinea fowl, which are used for their meat and eggs (Ramtahal et al., 2022). Poultry processing is mainly dominated by processors of exotic broilers (Kunadu et al., 2020; Oloo, 2017). In formal processing the chickens are received in the reception area at the slaughterhouses, inspection is often carried out to check general health and any manifestations of pests. In informal processing, chickens are kept in cages with a capacity of around 20-50 chicken each; there is no inspection to check the health of the chicken, which contributes to the slaughter of sick animals (Birgen et al., 2022) . Poultry processing in many countries around the

world and in Africa includes slaughter, scalding, plucking, gutting, cutting and, in some cases, deboning and/or grinding (Garsow et al., 2022).

However, several surveys on chicken slaughtering and processing in African countries report poor chicken handling practices, posing a risk to public health and the environment. In addition to contamination of chicken during processing, individuals involved in poultry processing can be exposed to foodborne pathogens as well as other zoonotic diseases (Garsow et al., 2022). Smallholder farmers in particular often do not have access to hygienic slaughter facilities, which can lead to cross-contamination of carcasses and the surrounding environment (Abdel-Naeem et al., 2022; Garsow et al., 2022).

During poultry processing many microorganisms that cause foodborne illness can spread at any stage of the processing chain but slaughter is most often described as the key step where microbes can be introduced from slaughterhouses, the environment, equipment and water of washing (Adejoh and Tanko, 2018). However, it is important to maintain good hygiene practices at this stage to ensure the safety of the chicken and to avoid the transmission of pathogens through food. Mpundu et al. (2019) reports unhygienic practices and bacterial contamination of chicken carcasses due to poor practices during processing; the presence of pests and flies, lack of appropriate clothing and the use of impure water to wash the carcasses are the main factors for cross-contamination in the chains of slaughter, sealing and consumption of chicken.

Birgen et al. (2022) points out another concern in relation to the processing of chicken in unhygienic conditions mainly in markets, the handling and environmental conditions can aggravate the risks of foodborne illnesses, as the carcass is exposed to contamination during slaughter and post-slaughter through from improper handling and temperature abuse. The informal sector in some sub-Saharan African countries has a weak legislative framework to address food security weaknesses in meat value chains compared to formal food retail and distribution chains.

The study carried out by Assefa et al. (2023), the result showed that the hygiene of the Market under study is bad, 86% of the slaughter places do not have adequate flooring, it is not washed regularly, 92% of the chicken are slaughtered on dirt floors; bleeding, plucking and gutting are done on a wooden table that is rarely washed, in general most operators washed their hands and knives with tap water at the beginning of the slaughtering process only. It was observed that about 33.8% of the interviewees washed their knives at the beginning of the slaughter and at the end of the day, while about 9% washed them after each batch of chickens, 16% washed their hands at the end of the slaughter. Most respondents 31% used tap water to wash the knives, followed by carcass rinse water 15.4% and then storage water 17%. With regard to garbage, 52% throw solid waste from slaughter outside the market and 11% prepare the waste to be taken by garbage collection services.

The same result was found by (Mokgophi et al., 2021) , whose poultry meat from formal slaughterhouses was not much safer than meat purchased from live poultry markets and farms that use informal slaughter processes. In addition Garsow et al. (2022) noted that waste litter is buried on home property or fed to dogs, which can cause contamination of animals, the environment, including contamination of drinking water. The authors report that in developing countries the risk of eating poultry meat from formal and informal small-scale producers, processed in formal slaughterhouses and informal slaughter points is unknown but diarrheal diseases remain an important cause of mortality. The authors propose the use of Hygiene Management Systems (HMS) and Hygiene Assessment Systems (HAS), using an adequate auditing system adapted to formal or informal processing as a way to guarantee food safety.

During the scalding phase Assefa et al. (2023) observed that an average of 33 chicken use the same scalding water, while seven chicken are scalded at the same time in the scalding tank, the scalding water was changed once a day, the processors also shared the slaughter site. To monitor contamination at this stage, it is recommended that the scalding water be continuously replaced throughout the day and also that the water temperature be monitored. According to Ogutu et al. (2019), the survival of *Enterobacteriaceae*, *Salmonella* and aerobic mesophiles is greater at low scalding temperatures and the continuous overflow of contaminated scalding water and the simultaneous introduction of fresh water will remove contaminants from the scalding tank water and prevent excessive bacterial accumulation in the scalding tank, in addition to the destruction of some bacteria by the thermal process.

Additionally, Assefa et al. (2023)verified that most of the interviewees use a wooden table 70.7% as a plucking surface and after removing the feathers, most processors 23% dispose of the garbage in trash cans or dump them in the street or market 20% or a combination of the two 17%. The authors noted that during plucking there is considerable dispersion of microorganisms from carcass to carcass and also from the plucking equipment itself. The other aspect of defeathering hygiene is the nature of the machines themselves and their placement close to the scald tank, which helps to maintain a warm and humid environment suitable for microbial growth. It is therefore necessary to ensure complete separation of the plucking and scalding area from the clean processing areas (Birgen et al., 2020).

The carcass processing step that occur more cross-contamination after plucking is evisceration. Most respondents 49%, pluck and eviscerate in the same place (Assefa et al., 2023). Among the worrisome practices reported by the authors is that 50% of

processors said they throw solid waste from slaughter and processing on the ground, outside the market and down the drain. According to Birgen et al. (2020), the high-rate processing of the carcasses on the line allows them to be close together and cross-contamination occurs readily, allowing little opportunity to sanitize equipment between carcasses. However, the viscera must be removed quickly through a relatively small opening in the abdomen to prevent contamination of the entire carcass.

In the markets, the chicken is slaughtered and blanched in hot water, after which the carcasses are plucked and eviscerated mainly by hand. Before and after evisceration, broiler carcasses are subjected to manual washing, which can spread bacteria from localized sites to the rest of the carcass, as well as between carcasses and all parts of the chicken carcass can be equally contaminated (Assefa et al., 2023). Many studies report that during evisceration there is an opportunity for *Enterobacteriaceae* contamination of intestinal contents. Cross-contamination can also occur due to workers' hands and evisceration.

Careless manual opening of the abdomen and manual evisceration lead to contamination of carcasses, particularly when the intestines are torn apart or the cloaca is loosened improper (Mokgophi et al., 2021). The washing phase reduces the count of mesophiles, *Enterobacteriaceae* and coliforms, the incidence of *Salmonella* in the carcasses also decreases after washing, but inadequate cooling can provide an increase in psychotropic bacteria. However, spoilage in cold temperatures is of greatest concern because *Pseudomonas* generally predominates in chicken and these bacteria easily recover and multiply at low temperatures (Muchangos, 2012).

According to Assefa et al. (2023) the manual evisceration technique practiced in the studied slaughterhouse, infrequent hand washing, poor personal hygiene and insufficient cleaning of equipment such as knives resulted in increased bacterial loads. Cross-contamination also occurred through contact between the carcasses in the processing line and through the friction of the carcasses against the taps of the evisceration troughs. The author highlighted that the survey showed that the processing steps where more bacterial contamination occurred were plucking and evisceration.

The lack of hygiene during processing was observed by Assefa et al. (2023) in which the workers did not wash their hands, which may have contributed to cross-contamination of the carcasses and a possible source of the high level of *Staphylococcus* that it obtained in this stage of processing. The author recommended that, in future studies, workers' hands be tested for *Staphylococcus* counts to establish the extent of contamination. Sumbana et al. (2022) study also revealed that cross contamination by *E. coli* and *E. coli* O157 occurred more in the cooling tank at Slaughterhouse B, due to mixing of carcasses inside the tank after evisceration and first washing, the carcasses were stained with blood and some stools due to the low pressure of the showers.

However, preventing rupture of the intestines and subsequent contamination of the carcasses during evisceration contributes to the reduction of bacterial contamination, in particular, *Enterobacteriaceae*. According to Oloo (2017) *E.coli* belongs to the large family of *Enterobacteriaceae*, reside in the intestinal tract, are found in large amounts in the feces and their presence demonstrates possible contamination with dirt or fecal material and must be controlled due to the possibility of food poisoning. In this study found a prevalence of *E. coli* in chicken samples 12.2%, while in plucking and evisceration and post-mortem area it was 15.8 and 7.7%, respectively. For *S. aureus* the prevalence in the post-mortem sections and evisceration in the slaughterhouse of 10%, the results showed a prevalence rate of around 15% of the different surfaces and rinse waters. As a microorganism commonly found in the nasal passages, throat and skin of carcasses, the key to dealing with *S. aureus* infections is to ensure hygiene during food handling at all stages from production to consumption because humans are a major source of *S. aureus* poisoning.

Eliminating manual carcass cleaning and spray washing should also prevent high prevalence of bacteria such as *S. aureus*, it will also prevent cross-contamination and transfer of microorganisms from one stage to another (Birgen et al., 2020; Sumbana et al., 2022). The author recommends that the air cooler be turned on punctually to ensure that the temperature in the cooler is - 6°C before the start of slaughter. In informal processing, it is possible for carcasses to be contaminated by animals such as rats, cats and dogs roaming around in search of food. Assefa et al. (2023) had the result of respondents in 26.0% who reported rats, 39.0% reported cats and 30.0% reported that dogs approached food preparation surfaces. Only 10.0% of respondents have control measures such as; fly sprays and other insecticides, poisons, traps and other processors prefer to cover carcasses tightly to prevent access.

Assefa et al. (2023) found that at chicken processing points 59.0% of processors are not licensed and 63.0% are not regularly inspected, the informal sector is dominant in Burkina Faso and largely unregulated. Similar is reported by Bamidele et al. (2022) in relation to food safety, that the standards are poor and the infrastructure so limited that major improvements would require massive investments to improve facilities and train slaughterhouses and other value chain actors, combined with an adequate level of regulation. Additionally, the authors suggest interventions such as training handlers in good hygiene practices, use of clean water and quality utensils.

SOURCES AND MECHANISM OF CONTAMINATION OF POULTRY

Food contamination in most developing countries is the result of several factors, such as methods applied in production, transport, food processing, and inadequate storage temperatures and poor personal hygiene of food handlers (Tshipamba et al., 2018). During production, poultry are often contaminated by various factors such as the environment in which they are found in the case of inadequate hygiene, the feed may contain pathogenic microorganisms if not protected, contaminated water and contact with contaminated animals.

According to Otwey and Kunadu (2022), the main sources from which poultry can obtain *Salmonella* are cross-contamination during rearing, incubation and intensive rearing operations and also when there is inadequate cleaning and disinfection of the multi-cage transport trucks used to take the chicken to the slaughterhouse. However, *salmonella* is not part of the normal intestinal microflora of poultry, but is acquired from the farm environment through insects, rodents and chicken. Feed is also a major source of *Salmonella* through contamination of various feed mix components. *Salmonella* from one batch can contaminate another during intensive rearing and live chicken transported from the farm can introduce *Salmonella* into the processing plant.

The study carried out by Malong (2022) in Uganda revealed the contamination of chicken feed by pathogenic microorganisms *Citrobacter* spp. (4.8%), *Corynebacterium* spp. (9.5%), *E. coli* (2.4%) and *Enterococcus* spp (35, 7%), *Proteus* spp. (2.4%) and S. aureus (45.2%). The presence of a high level of pathogenic microorganisms suggests that feed is a source of contamination for poultry. Therefore, poultry feed should be stored in secure spaces and assessed for biosafety before use to prevent cross-contamination.

According to Phosa et al. (2022) there is an intrinsic connection between the availability of clean water and safe food because water is used in the production, processing and preparation of food; contaminated water can easily affect the food supply. Additionally, Neri et al. (2019) considers that the mode of transporting chicken to the point of slaughter with poultry hanging together on motorcycles or other vehicles not only causes stress to the poultry, but also contributes to increased spread of pathogens and animal suffering; close contact between chicken increases cross-contamination with faecal microbes such as *Campylobacter* species. During unloading, some chicken will struggle and flap their wings, especially when hanging from the shackles, which results in a considerable scattering of dust and micro-organisms.

In Mozambique, retail outlets are rarely included in city plans and waste processing and collection facilities are not available (Muchangos, 2012). However, the waste produced can endanger the health of the consumer as it is a source of contamination for food products. At points of sale Assefa et al. (2023) observed the presence of rodents and other mammals, such as cats, dogs and goats, many stressed and with health problems, close to food preparation areas, creating a favorable environment for the transmission of diseases. Furthermore, it was also observed that the shed where the poultry are kept before slaughter is not frequently cleaned, creating conditions for the development of zoonotic pathogens that can contaminate the chickens and, in turn, consumers.

However, unhygienic management practices in markets can be a critical point for the possible emergence of new pathogens. In African countries, knowledge of good poultry slaughtering and processing practices is limited, mainly in the informal sector, the products processed and sold in these places are not always of good quality and the dangers arise from the lack of clean water for food preparation, washing of the processors utensils and personal hygiene (Assefa et al., 2023).

Water is one of the main potential sources of contamination of processed foods in Africa, either because of poor quality at the source or because it is subsequently dirty and misused. Chlorine-based disinfectants are most commonly used to destroy bacteria in water around the world using a process known as chlorination. In the informal slaughter Cambaza dos Muchangos (2012) observed that some processors used borehole water of unknown quality to process chicken, well water is not protected which is conducive to contamination by pathogenic microorganisms. Also found that in markets and on farms, water for scalding and washing is used several times and ends up having an excessive microbial load that contributes to the contamination of chicken. Good hygiene requires the abundant use of water for frequent washing of hands, knives, facilities and raw materials.

According to Adejoh and Tanko (2018) the conditions of environmental sanitation and the poor hygiene of food handlers are responsible for food contamination, while inadequate storage contributes to the multiplication of pathogenic agents in food in infectious doses. However, in underdeveloped countries with few resources, such as in sub-Saharan Africa, food is not properly packaged.

Slaughter and dressing are also the main sources of contamination, especially plucking, which is removing the feather, can contaminate the workers hand and, when performed together with evisceration, can contaminate the carcass (Assefa et al., 2023). In the environment of poor hygiene, raw chicken meat is an ideal substrate for the growth of pathogenic bacteria *E. coli* and coliforms, indicating the presence of other pathogenic bacteria such as *salmonella* and *Campylobacter*; this can be a major source of foodborne illness (Birgen et al., 2020).

Poultry are an important reservoir of *Campylobacter jejuni*. Contamination of poultry that are negative prior to slaughter can occur during processing through cross-

contamination or the processing environment not being properly disinfected (Ogutu et al., 2019). Several studies report that spoilage bacteria originate from the rearing environment and these organisms settle on the feet and feathers of chicken and another source of spoilage bacteria is the water supply of the processing plant.

According to Birgen et al. (2020) the source of spoilage organisms is mainly the rearing environment, the spoilage bacteria of poultry stored in aerobic conditions grow faster when the temperature is similar to that linked to their optimal growth (25°C). Inadequate control of food temperature is one of the most common causes of food spoilage. Food spoilage is wasteful, expensive and can adversely affect trade and consumer confidence. A similar analysis is made by Adejoh and Tanko (2018); Amoako et al. (2020), points out that the main contributing factor to outbreaks of *staphylococcal* food poisoning is inadequate control of cold temperatures, with initial contamination often attributed to poor personal hygiene by food handlers. The results of their study reveal that most *S. aureus* isolated from poultry processed in factories and farms produced *staphylococcal* enterotoxin D and *staphylococcal* enterotoxin A.

According to Birgen et al. (2020) the presence of *Staphylococcus* in chicken carcasses and at the point of sale indicated the presence of poor hygiene practices and food handling, cross contamination, associated with discharges from human clothing, human skin, dirty hands, mouth, nose and utensils. The same was reported by Oguttu (2015) that *S. aureus* is transmitted to food by food handlers or by cross-contamination through utensils previously contaminated by humans; food is usually contaminated during or after processing.

During manual evisceration at the slaughterhouse, Sumbana et al. (2022) observed the rupture of the intestines which resulted in high levels of faecal contamination, also found that the scalding and washing water of the carcasses was not renewed, factors that contribute to contamination by faecal material and the lack of drainage of wastewater that can serve as a source of contamination in the processing chain. During observational visits to markets and farms, also noted the lack of toilets and washbasins, in markets there were no facilities for bathing or changing clothes, vendors wore the same clothes they used to go to the market and return home. In the scalding phase, microorganisms from skin and feathers and chicken feces are washed from the poultry and continuously released into the water in the scald tank.

During plucking, there is an increase in aerobic mesophiles (*Micrococcus*, *Streptococcus* and *lactobacilli*), *S. aureus* and *Enterobacteriaceae*, due to a considerable dispersion of microorganisms inside plucking machines and inadequate maintenance and cleaning of plucking machines. *Enterobacteriaceae* counts increase during evisceration, *Salmonella* counts also increase after exposure and opening of the intestines (Assefa et al., 2023).

Studies indicate that infrequent hand washing, lack of personal hygiene by workers and insufficient cleaning of equipment, such as knives, led to a greater accumulation of bacteria. Essentials to avoid excessive levels of contamination include immediate washing and cooling of eviscerated chicken and effective cleaning and disinfecting procedures for equipment and work surfaces at the end of the processing day, prior to the next day production.

MICROBIOLOGICAL QUALITY AND SAFETY OF POULTRY PROCESSING IN AFRICA

The current food security situation in many developing countries in Africa, South Asia, Central and South America is worrying; it is very important to analyze the quality and microbiological safety of food to ensure the supply of safe food to the population of these regions (Akhtar et al., 2014). The microbiological quality and safety of exotic chicken and chicken products processed in Africa are summarized in Table 1.

In Africa, South Africa, Kenya, Nigeria, Morocco, Egypt and Algeria are the main countries in the production of chicken meat (Ramtahal et al., 2022). However, many studies carried out in African countries report insufficient information regarding the epidemiology of infection by *Campylobacter* and *Salmonella*, contamination by *coliforms*, *E. coli* and *S. aureus* (Birgen et al., 2020; Odwar et al., 2014; Ogutu et al., 2019; Oloo, 2017). According to Ramtahal et al. (2022) in Africa, there is little information on the occurrence of non-typhoidal serotypes of *Salmonella* enteric, therefore, they are a common cause of infections in poultry, but there is a lack of coordinated national epidemiological surveillance systems.

Poultry meat is highly nutritious; it provides a good medium for the development of microorganisms, some of which are pathogenic (Abdel-Naeem et al., 2022). The most common pathogenic microorganisms associated with chicken meat are *Listeria* spp., *E. coli*, *Salmonella* spp., *Staphylococcus* spp. and *Campylobacter* spp, zoonotic agents implicated in animal and human infectious diseases (Birgen et al., 2020; Muchangos, 2012; Oloo, 2017). Poultry contamination occurs at any stage of the production, processing and preparation process, contamination occurs mainly during slaughter, with additional exposure due to handling and preparation (Assefa et al., 2023; Birgen et al., 2020; Cambaza dos Muchangos, 2012).

Oloo (2017) reported a prevalence of *E. coli* of 15.9%, of *salmonella* of 24.4% and of *S. aureus* of 12.2%. This sturdy found in the plucking section a prevalence of 15.8% of *E. coli*, *Salmonella* 32.0% and *S. aureus* 10.0% and in the post-mortem area he had a prevalence of E. coli of 7.7%, *Salmonella* of 23.0% and *S. aureus* of

Table 1. Microbiological quality and safety of exotic chicken processing in Africa.

Country	*E. coli* (%)	*S. aureus* (%)	*Salmonella* (%)	*Campylobacter*	Samples type	References
South Africa	-	-	-	23.4	Fresh meat	Phosa et al. (2022)
South Africa	-	-	94.9	-	Fresh meat	Mokgophi et al. (2021)
South Africa	-	31.3	-	-	Fresh meat	Amoako et al. (2020)
Ethiopia	-	-	14.6	-	Chicken caecal	Asfaw Ali et al. (2020)
Nigeria	34.2	-	57.5	-	Poultry droppings	Bamidele et al. (2022)
Zambia	55.0	-	2.5	-	Fresh meat	Mpundu et al. (2019)
Mozambique	63.0	-	-	-	Fresh meat	Cambaza dos Muchangos (2012)
Mozambique	52.0	-	-	-	Fresh meat	Sumbana et al. (2022)
Ghana	-	-	61	18	Fresh meat	Kunadu et al. 9(2020)

Values are the percentage of sample containing bacteria**.**

15.4%, pathogen values were beyond hygienically acceptable standards. In this study, S. *Typhimurium* and S. *dysentery* were also isolated, with a prevalence of 32.0%. Infections by these species can lead to morbidity and deaths in both poultry and consumers and can affect a country's economy.

Adejoh and Tanko (2018) in the study of chicken sold on the streets of Lokoja in Nigeria found high levels of *Staphylococcus* spp., in the samples; he considers that it is indicative of human contamination after production, which may have occurred through human contact or indirectly through utensils. The organism is associated with endotoxin characterized by violent nausea, vomiting and diarrhea. In this study, also isolated *E. coli*, this associated contamination with the use of contaminated water during the processing stages, because water is the main means of propagation of *E. coli*, although some *Escherichia* spp are harmless.

A similar study in uMgungundlovu District, South Africa revealed high prevalence of all 384 samples cultured for *S. aureus* 120 (31.3%) were positive (Amoako et al., 2020). The reason for the high prevalence of *S. aureus* was attributed to poor personal hygiene of workers, the technique used to open the abdomen, manual evisceration and infrequent hand washing. Asfaw Ali et al. (2020) reported a high prevalence of *Salmonella* contamination of broilers 56 (14.6%) in Ethiopia. The high prevalence of *Salmonella* contamination in these regions highlights poor hygiene practices throughout the supply chain. Salmonellosis has been recognized in all countries, but tends to be prevalent in areas of intensive animal husbandry, particularly poultry production. Although primarily an intestinal bacterium, *Salmonella* is widespread in the environment and commonly found in agricultural effluents, human sewage and any material subject to faecal contamination. *Salmonella* is highly adapted to man and typhoid fever agents; highly adapted to animals responsible for animal paratyphoid and most serovars that affect humans and animals.

Bamidele et al. (2022) also found in Nigeria, through morphological and biochemical characteristics, a higher prevalence of *Salmonella* spp. (57.5%) and *E. coli* (34.2%) in chicken samples, the endemic nature of these species, the authors attribute to inadequate measures aimed at preventing and controlling the spread of infections, as well as poor hygiene, husbandry and biosecurity practices by part of the farmers.

Campylobacter jejuni, in Kenya was the most prevalent microorganism in raw and cooked poultry products, with 9.0±1.0 and 4.7±2.7 log CFU/g, respectively; compared to *Salmonella*, *E.coli* and *Staphylococcus* which showed contamination levels of 6.4±1.6, 6.6±1.3 and 6.9±1.3 log CFU/g in raw poultry respectively and 2.2±1.9, 2.7±2.0 and 2.9±1.6 in cooked poultry respectively (Ogutu et al., 2019). The author's report that in human *Campylobacter* spp. is associated with enteritis and gastroenteritis in adult and pediatric patients, a total of 30% of campylobacteriosis cases have been attributed to poultry consumption.

In the study carried out by Birgen et al. (2020) when evaluating the microbial contamination in chicken carcasses, the level of *E. coli* varied from 6.4 ± 1.6 to 2.2 ± 1.9; *Salmonella* spp., 6.4 ± 1.6 to 2.2 ± 1.9; *Staphylococcus aureus*, 6.9 ± 1.3 to 2.9 ± 1.6; and *Campylobacter jejuni*, 9.0 ± 1.0 to 4.7 ± 2.8 log CFU/g in raw and cooked chicken samples, respectively. The *E. coli* counts are associated with the presence of flies, the place of sale full of garbage, non-existent hand washing by the sellers and lack of adequate clothes for the protection of the food.

A study carried out in Mozambique by Muchangos (2012) revealed that the rate of contamination with *E.coli* type I was higher in informal markets (63.0%) than in carcasses from the formal slaughterhouses (33.0%).

Sumbana et al. (2022) found high levels of *E. coli O157* in chicken carcasses at different stages of processing in two Mozambican slaughterhouses. *E. coli* contamination was related to faecal contamination, due to the lack of ablutions in live animal markets, as well as the fact that chicken become very dirty with feces when they are

stacked on top of each other in cages. Sumbana et al. (2022) points out that the problem of consumption of chicken meat contaminated by *E. coli* and *E. coli O157* in Mozambique is considerable due to the lack of routine tests.

A food safety surveillance system must be designed to allow consumers to be protected against the health risks associated with foodborne pathogens such as *E.coli*, *S. aureus*, *Salmonella* and *Campylobacter*, using the technique of cultivation and growth on enrichment media, including isolation, biochemical, serological and subspecies characterization, DNA and antibody based testing, combining various methods, can improve the diagnosis of microbes in poultry meat (Birgen et al., 2020; Ramtahal et al., 2022).

The surveillance system should involve various entities (ministry of health, ministry of agriculture, food safety auditors, industries, universities, farmers and consumers) to decide on regulations and policies that should be implemented for monitoring, reporting and analysis of pathogens (Oloo, 2017; Ramtahal et al., 2022). Regular monitoring and audits should take place along the entire production chain from farm to consumption to identify pathogens. Detection and counting of microbes are important in food quality and safety control. The numbers of microbes and species identification define the safety or acceptability of food for human consumption.

PATHOGENIC AND SPOILAGE MICROORGANISMS ASSOCIATED WITH POULTRY IN AFRICA

Food contaminations in the meat value chains of sub-Saharan African countries are increasingly attributed to microbial risks rather than chemical risks (Birgen et al., 2020). Poultry meat can be contaminated by a large number of microorganisms, including food spoilage during refrigerated storage and even food-borne pathogens (Oloo, 2017). Pathogens associated with poultry that have already been isolated in African countries are *Salmonella, S. aureus, Clostridium perfringens* and *E. coli, L. monocytogenes* and *C. jejuni*. Additionally, there are spoilage bacteria associated with poultry are *Pseudomonas* spp., *Acinetobacter*, *Moraxella*, *Alteromonas putrefaciens*, *Aeromonas* spp., *Corynebacterium*, *Flavobacterium*, *Micrococcaceae* and *Enterobacteriaceae* (Asfaw et al., 2020; Oloo, 2017).

The *Enterobacteriaceae* family is a group of bacteria used to assess the general hygiene status of chicken and other foods and has been found to be a member of the microbial association implicated in the deterioration of food products during refrigerated storage (Abdel-Naeem et al., 2022; Asfaw et al., 2020). Processed and unprocessed poultry can harbor spoilage microorganisms, as well as food-borne pathogens; these microorganisms can contaminate poultry meat.

Spoilage microorganisms such as psychotropic have the ability to multiply in refrigerated storage, resulting in off-flavors. To determine the shelf life of the product, the count of deteriorating microorganisms present initially is made as well as the temperature history of the product in all stages of production, subsequent storage and handling, this analysis is important to guarantee the quality of the products (Abdel-Naeem et al., 2022; Oloo, 2017).

Spoilage microorganisms are mostly found on the skin of poultry and mammals. Humans are a vehicle for transmission of *S. aureus*, it can be found in the nasal passages, throat and skin of carcasses. Poultry meat is associated with the presence of food-borne pathogens such as *Campylobacter*, *E. coli*, *Salmonella* enteritidis and *S. aureus*, especially when processing conditions are not hygienic (Birgen et al., 2020; Oloo, 2017). Many studies report that *Salmonella* and *Campylobacter* are associated with poultry contamination, but there are *Flavobacteria* and *Pseudomonas* known to cause food spoilage. *Flavobacteria* are in the form of yellow pigmented rods, originating from chicken or the processing environment (Muchangos, 2012; Oloo, 2017).

According to Abdel-Naeem et al. (2022) the main source of spoilage organisms is the poultry rearing environment and they are brought into the plant from outside the chicken. Generally, aerobically stored poultry spoilage bacteria are pigmented and non-pigmented strains of *Pseudomonas* spp., which grow most rapidly when the temperature is similar to that associated with optimal growth (25°C). It is recommended to control the temperature of food to prevent the growth of these microorganisms, which contribute to waste and expense and which can negatively affect trade and consumer confidence in the event of food contamination.

Another source of spoilage bacteria is also the processing plant water supply, which contributes to the prevalence of *Pseudomonas* in the processing area is that they are described as more resistant to chlorine than *E. coli* and can survive normal water treatment at the processing plant. Therefore, the quality of water in the processing plant is of importance and to avoid excessive levels of contamination, immediate washing and cooling of eviscerated poultry and effective procedures for cleaning and disinfecting equipment, work surfaces at the end of the processing day, and before the next day production (Adejoh and Tanko, 2018).

Oloo (2017), reported a 17.6% prevalence of *Pseudomonas* the counts were highest in the dip tank samples. Contamination by *Pseudomonas* was associated with process water, because these are bacteria that easily multiply on the wet and dirty surfaces of the equipment and on the evisceration chute itself. In laboratory analyses, high counts of mesophilic aerobic bacteria indicate the presence of spoilage and there is a relationship between the proliferation of these microorganisms with time and temperature.

Muchangos (2012) noted that pseudomonas

predominated in deterioration when poultry carcasses were kept at 1°C, also found a high frequency of mesophilic aerobic bacteria and *E. coli* Type I in live chicken on the market, which attributed to the place where the chicken are stored, due to high ambient temperatures and the lack of a cold chain. *E. coli* Type I is related to faecal contamination in that chicken become heavily soiled with feces when they are stacked on top of each other in cages.

The risk of contamination of poultry carcasses is greatest during processing and can only be avoided by using strict food safety management systems. Oloo (2017) propose the routine surveillance of the most potent pathogenic microorganism in the poultry production and supply chain, targeting *Escherichia coli*, *Coliforms*, *Salmonella*, *Staphylococcus aureus* and *Campylobacter*. Another study carried out by Sumbana et al. (2022) used the 3M™ Petrifilm coli/Coliform Plate method, 74.0% samples from slaughterhouse A and 58.0% samples from slaughterhouse B, the result was considered unacceptable. For *E. coli* O157, 30.0% of the samples from slaughterhouse A and 24.0% of the samples from slaughterhouse B were positive. The study revealed that the lack of good food handling practices was the main reason for the high level of contamination in slaughterhouses.

Salmonella is a genus of rod-shaped, facultative Gram-negative bacteria in the Enterobacteriaceae family (Kunadu et al., 2020; Mokgophi et al., 2021) . They are facultative anaerobic, do not ferment lactose, do not form spores, and most are motile. In many African countries *Salmonella* is recognized as the most important food-borne pathogen associated with poultry meat, the extent to which consumption of poultry meat is responsible for human salmonellosis is currently unknown but most infections are zoonotic and are transmitted from animals to humans through contaminated food. *S. Typhi* is of human origin and can contaminate food during primary production via sewage as a result of poor personal hygiene.

S. enterica serovar typhimurium is prevalent in chickens but is transmitted by rodents and causes enteric diseases in many animals and also in humans. This pathogen colonizes chicken during growth, skin and meat carcasses are contaminated by pathogens during slaughter and processing but can be found in poultry litter and can be a source of contamination for newly arrived chicks. *Salmonella* also multiplies if the chicken is stressed, and elevated levels in carcasses can be associated with poor transport and slaughter practices that increase stress in the poultry (Muchangos, 2012).

A systematic review of the literature and meta-analysis on poultry contamination with *Salmonella* and *Campylobacter* spp., in 27 African countries, *Salmonella* was isolated in 3,615 poultry samples and 2,236 (61.9%) were serotyped and 464 (20.8%) were Salmonella enterica serovar Enteritidis, 311 (13.9%) were *Salmonella* enterica serovar Typhimurium and 174 (7.8%) were *Salmonella* enterica serovar Typhi, the prevalence was estimated at 24.6% of *Salmonella* and 13.1% of *Campylobacter* (Thomas et al., 2020).

Campylobacter is highly disseminated in the environment, is frequently isolated from wild and domestic animals, chicken is a reservoir of *Campylobacter* and are implicated as a vehicle for infection in humans. *Campylobacter* is microaerophilic with a relatively high minimum growth temperature (30°) (Phosa et al., 2022). Kouglenou et al. (2020) in the study found a 32.8% contamination rate of Campylobacter in the samples. Of which 59.5% were local chicken legs and 40.5% were imported chicken legs. Molecular identification in 256 samples revealed a prevalence of *C. jejuni* of 23.4% and of *C. coli* of 7.8%.

Many studies recommend the training of handlers to ensure changes in behavior and to avoid the accumulation of bacteria during processing. The following steps can help to reduce contaminations: continual changing of contaminated scalding water and introduction of clean water, thorough and frequent washings and rinsing, especially during plucking and gutting. High pressure spray washers after opening the carcass and after removing the intestines during the evisceration process will also reduce the number of bacterial contaminants. This should be combined with frequent rinsing of the plucking machines with clean water and a disinfectant, installing a countercurrent system during scalding to ensure that the carcasses come into contact with the cleanest water as it exits the scalding tank will reduce the accumulation of bacteria.

OCCURRENCE OF ANTIMICROBIAL RESISTANCE AND ANTIBIOTIC RESIDUES

Poultry production has been identified as a hot spot for the development of antimicrobial resistance and the transfer of drug-resistant microorganisms between food-producing animals and humans. The spread of antibiotic-resistant bacteria from animals for human consumption and the environment poses a particular risk to humans due to possible treatment failure (Bamidele et al., 2022; Kimera et al., 2020; Oloo, 2017). The use of antibiotics in poultry farms helps to reduce mortality, reduce the incidence of diseases, improving the health of the animals and, most importantly, increasing productivity. Some of the antimicrobial resistant bacteria reported in poultry production in Africa are summarized in Table 2.

According to Akinwumi et al. (2017); Mak et al. (2022), in chickens, antibiotics are administered in drinking water or in the feed to control the intestinal flora, as a way of preventing the appearance of infections and the misuse of nutrients provided by the feed. Additionally, the authors observed that with the use of antibiotics, there was growth in the chickens and an increase in the feed

Table 2. Antimicrobial resistant bacteria reported in poultry production in Africa.

Country	*E. coli*	*S.aureus*	*Salmonella*	Campylobacter	Resistant genes	Sample type	References
Nigeria	19.5% pefloxacin and 4.9% streptomycin	-	20.3% nalidixic acid and 8.7% ciprofloxacin	-	-	Poultry droppings	Bamidele et al. (2022)
South Africa	-	Tetracycline (61.7%), penicillin (55.8%), erythromycin (54.2%), clindamycin (43.3%)	-	-	*nuc* gene	Poultry meat	Amoako et al. (2020)
Ethiopia	-	-	Oxytetracycline (82.0%) and ampicillin (70.0%)	-	-	Chicken caecal	Asfaw Ali et al. (2020)
Benin	-	-	-	Ciprofloxacin (71.4%), Ampicillin and tetracycline (19.5%)	-	Chicken thighs	Kouglenou et al. (2020)
Ghana	-	-	Ciprofloxacin (22%) and Chloramphenicol (9%)	-	-	Chicken meat	Kunadu et al. (2020)
South Africa	-	-	Aminoglycosides, penicillin, sulfonamides, fluoroquinolones and tetracycline	-	aac(6¢)-Iaa, aacC, aadA, aph(3¢)- Ia, and estX ,sul and tet genes	Livers of chickens	Ramatla et al. (2017)

conversion yield, which resulted in weight gain. The indiscriminate use of antibiotics in poultry has led to the development of antimicrobial resistance in foodborne pathogens; it is also the main cause of antibiotic residues in edible tissues, liver and kidneys (Bamidele et al., 2022).

In many developing countries, levels of antimicrobial resistance tend to increase, due to lack of control by authorities, indiscriminate and widespread use of antimicrobials, both in veterinary and public health practices and also access to antimicrobials is easy because it can be purchased without a prescription (Amoako et al., 2020; Asfaw et al., 2020). In South Africa, a study reported resistance to trimethoprim-sulfamethoxazole, in about 30.0%, was combined with the use of sulfonamides in agricultural systems, since sulfonamides occupy the third place of the most used antimicrobial in the production of animal feed (Amoako et al., 2020).

The indiscriminate use of antimicrobials without considering the withdrawal period poses a significant risk to public health in terms of drug residues and the transfer of resistant bacteria to humans. Withdrawal of the antibiotic before slaughtering the chicken is the solution to avoid the problem of antibiotic residues and is an acceptable standard practice to preserve health, however, it can be difficult to monitor if poultry farmers are following this guideline, since buy antibiotics without follow-up (Selaledi et al., 2020). Additionally, the use of antibiotics in food has been reevaluated because bacterial pathogens have adopted survival mechanisms, through the transfer of antibiotic resistance genes that can be easily disseminated within microbial communities (Selaledi et al., 2020). In Africa, some countries have introduced restrictions on the non-therapeutic use of antibiotics as a way to prevent

antimicrobial resistance. Namibia and South Africa are the first countries in Africa to ban the routine use of antimicrobials, governments have developed an action plan to monitor and reduce antimicrobial resistance (Founou et al., 2018).

Other's countries in Africa report having a strategic plan to reduce antimicrobial resistance. However, it is difficult to estimate the exact prevalence of antimicrobial resistance in Africa due to the low number of antimicrobial resistance surveillance programs (Founou et al., 2018). Although there is little information on the occurrence of antimicrobial resistance in Africa, some studies consider that its occurrence represents a threat to food safety and security (Medina et al., 2020).

In sub-Saharan Africa, tetracyclines, aminoglycosides, macrolides, penicillin, quinolones, sulfonamides, diaminopyrimidines and fluoroquinolones are the most commonly used classes of antibiotics to treat various diseases in poultry (Azabo et al., 2022; Ramatla et al., 2017). Studies indicate that Gram-negative bacteria, such as *Acinetobacter* spp., *E. coli*, *Klebsiella* spp., *Campylobacter* and *Salmonella* spp., are the most resistant to antibiotics (Selaledi et al., 2020; Bamidele et al., 2022).

On African continent there are few studies on the prevalence and antibiotic resistance of *S. aureus* infections in poultry due to lack of monitoring of this microorganism (Amoako et al., 2020). Few studies found during the review relate to the importance given to other microorganisms, such as *Salmonella*, *Campylobacter*, *Escherichia coli* and *Listeria*. However, the authors recommend including *S. aureus* in surveillance and monitoring schemes for foodborne bacteria that routinely contaminate food supplies on the continent.

In South Africa, a *S. aureus* prevalence rate of 31.3% was reported, the isolates were resistant to growth promoters and antibiotics used in veterinary and human medicine: tetracycline (61.7%), penicillin G (55.8%), erythromycin (54.2%), clindamycin (43.3%), doxycycline (36.7%), ampicillin (34.2%), moxifloxacin (30.8%), amikacin (30.8%), trimethoprim-sulfamethoxazole (30.0%) and levofloxacin (23.3%), the multidrug resistance and antibiotic resistance rates were 39.2 and 0.23%, respectively (Amoako et al., 2020).

The authors attribute the high rate of resistance to the intensive farming nature and the use of antibiotics as growth promoters, as well as in the treatment of infections. The authors recommend that effective antibiotic administration guidelines be in place to meet current requirements for prudent use of antibiotics in production, in conjunction with ongoing surveillance and monitoring schemes for S. *aureus* in food animals, in order to combat antimicrobial resistance in the continent.

In Uganda Malong (2022) reported resistance of *S. aureus* to gentamicin (79.0%), *Corynebacterium* spp., to linezolid (100%), *Enterococcus* spp., to gentamicin (100%) and rod-negative *enterococci* to cefoxitin (100%). The author relates this occurrence to the inappropriate use of antibiotics for the treatment and prevention of infections in poultry and as growth promoters in animal feed.

In Tanzania Mdegela et al. (2021) found in poultry contaminated with strains of *E. coli* resistant to amoxicillin + clavulanate, sulfamethoxazole and neomycin. They pointed out the excessive and improper use of antimicrobials in food and agricultural production that contributed to the emergence and spread of resistance. In Kenya a prevalence of antimicrobial resistant *E. coli* isolated from raw chicken was reported, 117 (75.0%) *E. coli* isolates were resistant to 12 antibiotics tested; resistance was highest for tetracycline, trimethoprim-sulfamethoxazole, ampicillin and streptomycin, 40.4% of the *E. coli* isolates were positive for the ten tested virulence genes.

Additionally, a study conducted in Nigeria analyzed 120 fresh chicken droppings, the prevalence of *Pseudomonas* spp., *Klebsiella* spp., *Salmonella* spp., and *E. coli* was 2.5, 5.8, 57.5 and 34.2% respectively. *Salmonella* spp. isolates were resistant to nalidixic acid. (20.3%) and ciprofloxacin (8.7%); E. coli showed the highest resistance to pefloxacin 19.5% and streptomycin 4.9%. Resistance of *Pseudomonas* spp., was observed in 33.3% for ciprofloxacin and cotrimoxazole and Klebsiella spp., showed greater resistance to penicillin 42.9%. The results suggest an abuse of quinolone antibiotics in poultry production (Ramatla et al., 2017).

A similar study conducted in Ethiopia, in a total of 384 samples of chicken cecal contents using the Kirby-Bauer disk diffusion method, found a prevalence of 14.6% *Salmonella*, with greater resistance to oxytetracycline (82.0 %) and ampicillin (70.0%), multidrug resistance was recorded in 43 (86.0%) of the isolates and the phenotype OXT, AMP, CHL, NAL, SXT and STR occurred more frequently (Asfaw et al., 2020).

The occurrence was attributed to the basic mechanisms of antimicrobial resistance, such as: modification of the antibiotic, decreasing the absorption or increasing the efflux of the antibiotic through the use of enzymes; change in the target site of the antibiotic; and acquiring the ability to break down or modify the antibiotic. The authors recommend adopting better hygiene practices on farms to reduce the occurrence of *Salmonella*.

In Southern Benin, 77 strains of *Campylobacter* were tested after PCR confirmation, 72.7% were resistant to ciprofloxacin, 71.4% were resistant to ampicillin and tetracycline, 19.5% of the strains were resistant to amoxicillin and clavulanic acid , 11.7% were resistant to erythromycin, 7.8% were resistant to gentamicin, and 55.8% of the strains were multidrug-resistant (Kouglenou et al., 2020). *C. jejuni* strains were more resistant to ampicillin and *C. coli* were resistant to tetracycline and erythromycin.

The result reveals that resistance to antibiotics is a

health problem, due to the symptoms of campylobacteriosis which are the same as those of gastrointestinal infections caused by other bacterial pathogens. The results were allied to the common and uncontrolled use of antibiotics in poultry farms to combat bacterial infections, which is why molecular characterization of resistance genes is imperative for a better understanding of genetic transmission and resistance mechanisms. In most African countries, it is difficult to control antimicrobial residues due to the lack of complex laboratory equipment and the high cost required to purchase reagents, which is why information on the occurrence of residues is limited (Lakew et al., 2022). However, antibiotic residues in meat are a serious public health problem due to their harmful effects on consumer health such as hypersensitivity, gastrointestinal disorders, tissue damage and neurological disorders (Ramatla et al., 2017).

A study conducted by Ramatla et al. (2017) in South Africa on the occurrence of antibiotic residues in meat samples using different analytical methods, the ELISA method illustrated that 56, 34, 18 and 25.3% of the sample tested positive for ciprofloxacin residues, streptomycin, sulfanilamide and tetracycline respectively. Using the TLC method, sulfanilamide showed 92.5% of positive samples, followed by streptomycin with 29.4%, ciprofloxacin 21.4% and tetracycline 14.6% while HPLC detected 8.3, 41.1, 88, 8 and 14.6% of samples containing residues, respectively, with ciprofloxacin and sulfanilamide.

Concentrations of antibiotic residues were in the ranges of 19.8–92.8, 26.6–489.1, 14.2–1280.8 and 42.6–355.6 g/kg with ELISA, while HPLC detected concentration ranges of 20.7–82.1, 41.8–320.8, 65.2–952.2, and 32.8–95.6 g/kg for sulfanilamide, tetracycline, streptomycin, and ciprofloxacin, respectively. Ciprofloxacin and streptomycin residue levels were above the recommended MRL limit of the Codex. A similar study also carried out in South Africa determined the presence of residues in liver samples, the limits of quantification and concentrations were above the maximum residue limit, doxycycline was detected in 24 (24.5%) of the 98 chicken livers, and 15 (15.3%) of the 98 were non-compliant. Concentrations of 919.0-6 1081.3 ppb and 1410.6-6108.9 ppb were obtained (Adesiyun et al., 2021).

In the systematic review Ramtahal et al. (2022) reported that *Salmonella Enteritidis*, *Salmonella Kentucky* and *Salmonella Typhimurium* serotypes were reported in all regions of Africa and antimicrobial residues were also detected in chickens was related to non-compliance with safety intervals or exceeding recommended doses. They also reported the resistance genes associated with aminoglycoside resistance [aac(6¢)-Iaa, aacC, aadA, aph(3¢)-Ia and estX], β-lactams: penicillin and cephalosporins (bla genes: CMY, CTM, CTX, OXA, PSE, SHV, and TEM), fluoroquinolones and quinolones [aac(6¢)-Ib-cr, gryA, parC, qnrB, and qnrS], phenicols (catA1, floR, and cmlA), sulfonamides (southern genes), diaminopyrimidines: trimethoprim (dfrA , dhfr, orfF and sul genes) and tetracyclines (tet genes). A summary of antibiotic residues reported in poultry production in Africa is presented in Table 3.

The presence of antibiotic residues in food is a concern, as it poses a risk to public health for humans and animals, such as toxicity and development of resistance. Prolonged exposure to doses of antibiotic residues in the body can lead to acute or chronic toxicity to organs and the whole body and can also cause allergic reactions or produce drug-tolerant bacteria in humans after long exposure. There is a need to encourage producers to respect the withdrawal periods for antimicrobial agents, in order to reduce the level of antimicrobial residues to a minimum and also to reinforce controls through regular sampling and analysis.

DETERMINANTS OF OCCURRENCE OF ANTIMICROBIAL RESISTANCE AND ANTIBIOTIC RESIDUES IN POULTRY PRODUCTION

In sub-Saharan Africa, poultry farmers play a key role in preventing the spread of resistant microorganisms and producing meat free of antimicrobial residues, but inexperienced and untrained breeders can contribute to the incidence and spread of antimicrobial resistance. Studies indicate that the factors that contribute to the indiscriminate use of antimicrobials in many African countries are the intensive system used in poultry production that favors the emergence of diseases, poor knowledge about the consequences of inappropriate use of antimicrobials, little experience about appropriate use of antimicrobials by smallholders and the lack of strict enforcement of regulations by governments (Kimera et al., 2020; Mak et al., 2022; Ramatla et al., 2017).

Most poultry farmers claim that it is impossible to raise poultry without the use of antimicrobials because the animals are often sick and the drugs are easily accessible, coupled with the lack of adequate monitoring for the use of antibiotics (Mak et al., 2022). Poultry farmers justify the use of antibiotics in poultry production to promote growth and prevent major diseases (Amoako et al., 2020; Mak et al., 2022).

In Tanzania, an interview was conducted with poultry farmers to find the relationship between knowledge and the use of antibiotics for disease prevention. The study revealed that there is a higher proportion of use of veterinary antimicrobials for disease prevention (87.6%) than for therapeutic purposes (80.5%) (Kimera et al., 2020). The high use of antimicrobials was attributed to the high frequency of occurrence of diseases and the easy accessibility of antimicrobials, due to the insistence and encouragement to poultry farmers by private sellers of veterinary drugs to use combinations of drugs in order to improve production.

Table 3. Antibiotic residues reported in poultry production in Africa

Country	Antibiotic residue	Level µg/g	Sample type	References
South Africa	Ciprofloxacin	89.6-175.9	Muscle	(Ramatla et al., 2017)
	Streptomycin	98.4-452.9		
	Sulphonamides	32.5-65.9		
	Tetracycline	41.2-82.1		
	Ciprofloxacin	152.2-289.1	Liver	
	Streptomycin	368.8-986.4		
	Sulphonamides	45.8-81.6		
	Tetracycline	42.7-286.2		
Ghana	Tetracycline	0.02	Kidney	(Johnson et al., 2019)
		0.01	Liver	
Kenya	Amoxicillin	113.36	Chicken meat	(Odundo et al., 2023)
	Ampicillin	102.2	Chicken meat	

The authors propose the implementation of interventions focused on optimizing the use of antimicrobials in animals and humans and the establishment of measures: to minimize environmental contamination, the occurrence of infections and use of antibiotics and to promote health and productivity to achieve the goals of sustainable development. The results of the systematic review conducted by Mdegela et al. (2021) reveal that farmers buy antimicrobial agents to treat animals without the prescription of animal health specialists, and the management of antibiotics is characterized by incorrect dosages, misuse, incorrect applications and non-compliance with withdrawal periods. Farmers reported that animal health professionals prescribed antimicrobials for prevention, growth promotion, and animals driven for slaughter in markets are stabilized with antibiotics during tracking to destination, which contributes to non-compliance with the resistance interval.

The most commonly used classes of antimicrobial agents in animals in Tanzania are tetracycline, sulfonamides, penicillin, macrolides and others, including antiprotozoal agents, in feed formulations tetracycline (chlortetracycline and oxytetracycline), colistin and neomycin are used. Neomycin-oxytetracycline is also given in water. The use of antibiotics in poultry production as well as in other production systems has a negative impact on the environment because with the application of animal manure, considered a good source of nutrients it contributes to the dispersion of antimicrobial agents resulting from therapeutic and subtherapeutic uses extensive.

In Zambia, Mudenda et al. (2022) conducted a cross-sectional study with 178 community pharmacy professionals using a semi-structured questionnaire, the results revealed that the most widely dispensed and used antibiotics in poultry production are oxytetracycline (without medical prescription), gentamicin doxycycline and amoxicillin. The authors found that pharmacy professionals have moderate knowledge, positive attitudes, and moderate practices regarding the use of antibiotics and poultry antimicrobial resistance. The distribution of antibiotics to poultry without a prescription has been confirmed, which requires strict implementation of antimicrobial management and surveillance programs in poultry production to reduce antimicrobial resistance.

The results of the study carried out in Nigeria revealed that the use of antibiotics for prophylactic and growth improvement purposes, the probability of using antibiotics increases with flock size, but the amount used per chicken is greater among smallholders and the indiscriminate use of antibiotics (Oloo, 2017). These results reveal that the contribution of small farms in potentially creating an antibiotic resistance problem in developing countries should not be ignored. The authors advise smallholders to work in farmers associations, along with extension agents and private antimicrobial traders, to raise awareness of the appropriate use of antibiotics in poultry production.

According to Mdegela et al. (2021), poultry farmers report difficulties in adhering to safety intervals because they negatively interfere with production due to high economic losses, when some poultry are not stabilized to support the journey from the farm to the slaughterhouses or markets, they lose their lives. Most studies recommend the implementation of good practices in poultry production involving pharmacy professionals, veterinarians and farmers, in order to promote behavior, change in relation to the handling and administration of antibiotics. For poultry farmers in particular, it is necessary to be trained and instilled in them that the

prudent use of antibiotics is an attitude that allows the production of safe food. Government and food safety experts are recommended to develop programs to monitor the use of antimicrobials along the food chain to mitigate antimicrobial resistance

POTENTIAL FOOD-BORNE ILLNESS OUTBREAKS ASSOCIATED WITH CONSUMPTION OF POULTRY

Ensuring food safety is a global challenge in most underdeveloped countries that are victims of the dangers of foodborne illness. However, the prevalence of foodborne illness continues to be a cause of morbidity and death in Africa, although it is not well documented because poor monitoring and surveillance is recognized as a potential determinant for the rapid decline in economic growth in underdeveloped countries(Abdel-Naeem et al., 2022; Adejoh and Tanko, 2018).

Foodborne illness poses a worldwide challenge, with over 250 types of foodborne illness currently described (Oguttu, 2015). Meat is the main vehicle for pathogens that cause foodborne illness and is implicated in foodborne outbreaks related to poor food handling practices. The African continent suffered major economic losses at 32% related to diarrheal diseases, showing a connection with foodborne illness and the failure to ensure food safety, compromising economic growth (Akhtar et al., 2014).

Poultry meat is the second most consumed meat in the world, but it has been identified as one of the most important vehicles of foodborne pathogens, particularly *Salmonella* spp. and *Campylobacter* spp, present in the intestinal tract of poultry, which often appear asymptomatic (Birgen et al., 2020; Khalid et al., 2020). The presence of foodborne microbial pathogens along the poultry meat supply chain is a major public health concern (Khalid et al., 2020).

In African countries most foodborne illness outbreaks stem from poor supply systems and microbiological contamination during food production, processing, storage and handling (Oloo, 2017). Failure to apply good hygiene practices during production and processing combined with sanitation are prevalent and are associated with poverty, low education levels and lack of support from the government (Kunadu et al., 2020; Oloo, 2017). Several studies in Africa report the indiscriminate use of antibiotics and the occurrence of antibiotic residues, which allows the spread of antibiotic-resistant bacteria from animals for human consumption and the environment, these reports pose a particular risk to humans due to a possible failure in treatment (Bamidele et al., 2022; Kimera et al., 2020).

According to Birgen et al. (2020) assessing the prevalence of foodborne illness in underdeveloped countries is the most neglected area for disease control. The author adds that botulism, shigellosis, campylobacteriosis, *E. coli* infection, *S. aureus* infection, salmonellosis, listeriosis, and cholera are widely prevalent and pose a major threat to human health in disadvantaged communities.

Most pathogens that cause disease in poultry are bacteria such as *E.coli*, *Salmonella* spp. and *Campylobacter* spp., the handling and consumption of poultry can lead to foodborne illness (Selaledi et al., 2020; Garsow et al., 2022). Additionally Oloo (2017) adds that poultry meat is one of the main sources of contamination by foodborne pathogens, such as *Campylobacter*, *E.coli*, *S.enteritidis* and *S.aureus*. There is evidence from public health records that foodborne illnesses due to eating contaminated food are on the rise.

Public health records show that Africa is one of the continents with the highest number of foodborne illnesses, with approximately 91 million related illnesses and 137,000 deaths per year, representing economic and labor losses (Selaledi et al., 2020). In East Africa the incidence of foodborne illness due to poultry has been estimated at 96 disability-adjusted life years or the amount of health years lost per 100,000 people, which represents at least twice the burden in all non-African regions of the world (Li et al., 2019). Additionally, the majority of foodborne illness cases in Africa related to the 31 foodborne hazards caused 1200-1300 disability-adjusted life years per 100,000 individuals, of which nearly 70% of cases are estimated to be due to *non-typhoidal Salmonella* and *enteropathogenic* and *enterotoxigenic E. coli* (Makinde et al., 2020).

Foodborne illnesses in Kenya, reports reveal that they were caused by *Salmonella*, S. aureus and *E.coli* (Oloo, 2017). Birgen et al. (2022) in Kenya found a 52.9% incidence of foodborne illness with diarrheal symptoms. The study conducted in South Africa reported a prevalence of *C. jejuni* at 90.3% and *C. coli* at 9.7%, found in diarrheal diseases (Samie et al., 2022). In Madagascar, gastroenteric diseases each year cause 37.0% of deaths and about 50.0% of children fewer than 5 years of age are infected with intestinal pathogens. Additionally, in Tanzania *Campylobacter* was reported to occur in 9.7% of 300 children under five who had acute watery diarrhea. In Mozambique, the report provided by the health information system reports that microorganisms such as *Vibrio cholera*, *Salmonella typhi* caused 160 and 17 deaths respectively, dysentery and diarrhea caused 20 and 782 deaths respectively, *E.coli* and *Salmonella* were the main bacteria isolated from samples from patients with diarrhea (Muchangos, 2012).

Sumbana et al. (2022) reported the presence of *E. coli* in chicken carcasses collected in Mozambican markets and slaughterhouses, the result was combined with the possible presence of intestinal pathogenic organisms such as *Salmonella* and *Campylobacter* which in other countries have proven to be the main causes of FBD. The author adds that strains of *E. coli* can cause severe, fatal diarrhea and the presence of bacteria of fecal origin

is an indicator of serious illnesses, such as typhoid fever and viral hepatitis.

Furthermore, pathogenic *E. coli* strains such as 0157:H7 can cause severe intestinal illness in man, symptoms include abdominal cramps, watery diarrhea, lower intestinal bleeding accompanied by vomiting, fever in extreme cases can cause hemolytic uremic syndrome or renal failure (Adejoh and Tanko, 2018; Sumbana et al., 2022). These diseases affect poor and vulnerable individuals, including children, the elderly and immunocompromised people.

In Africa, through a systematic review of the literature on the contamination of poultry with *Salmonella* and *Campylobacter* spp, the prevalence was confirmed, 24.6 and 13.1%, respectively, in 27 countries (Thomas et al., 2020). According to Oloo (2017) most food-borne illnesses were attributed to non-typhoid diarrhea, causing more than half of deaths in children; some cases were linked to poultry meat and its derivatives. However, in developing countries, many cases of disease go unreported, and when reports are made, tedious culture techniques sometimes lead to misdiagnosis or are too slow (Muchangos, 2012; Lakew et al., 2022; Oloo, 2017). Additionally, the literature review on the prevalence of *Salmonella* in Africa, the prevalence ranged from 12.1% in Zimbabwe to 100% in Egypt, Ethiopia, Nigeria, Senegal and South Africa. The use of antimicrobials was described in 11.5% of the studies and *Salmonella* multidrug resistance was reported in 30 studies.

In another literature review study carried out by Birgen et al. (2022), it revealed that some studies detected *Salmonella* in cooked chicken, on hands, knives, surfaces and containers, the results were attributed to improper processing, staff hygiene and cooking temperature. *Campylobacter jejuni* contamination in raw and cooked chicken was 9.0±1.0 log10 CFU/ g and 4.7±2.7 log10 CFU/g respectively, the result was considered a threat to food safety, as these microorganisms are pathogenic and can cause illness to consumers. Ogutu et al. (2019) in Nairobi, in cooked chicken samples found counts above the infectious dose of *C. jejuni* 4.7±2.7 log CFU, contamination was related to poor post-processing handling, which included contamination of display surfaces and hands. Poisoning by *S. aureus* occurs in the absorption of staphylococcal enterotoxins that are pre-formed in food, infections result in wounds or lesions in the mucous membranes, symptoms include nausea, profuse vomiting, abdominal pain and diarrhea (Oloo, 2017).

Amoako et al. (2020) also reports that in Africa there is limited data on microbial quality and safety in poultry production, in particular for *S. aureus*, also its prevalence and significance for human health is incomplete. However, the study conducted by the author revealed contamination of chicken carcasses by *S. aureus* in 31.25% (120) of the 384 samples. Bamidele et al. (2022) reported the contamination of chicken carcasses by *Salmonella* spp., *E. coli* and bacterial isolates, which were multiresistant to the antibiotics used in production. These microorganisms cause disease and may also contribute to horizontal transfer of resistance genes with implications for the diagnosis of species-specific bacteria and treatment failure in humans.

Salmonellosis is caused by *Salmonella* spp., Gram-negative enteric bacteria, causes human food poisoning, and is often implicated in chicken contamination. The serotypes *S. enteritidis* is the most prevalent and *S. typhi* and *S. paratyphi* are more dangerous in underdeveloped countries due to the availability of food prepared in conditions with low hygiene (Mokgophi et al., 2021). Salmonellosis can lead to more serious cases like septicemia and typhoid if the bacteria invade the lymphatic system.

Thermotolerant species of *Campylobacter* (*C. jejuni*, *coli*, *lari* and *upsaliensis*) are the cause of human campylobacteriosis. However, *C. jejuni* and *C. coli* are important food-borne pathogenic bacteria associated with poultry (Phosa et al., 2022). In humans they cause symptoms of gastroenteritis, a self-limiting diarrheal illness due to poorly prepared or contaminated food, including poultry products (Mak et al., 2022).

In developing countries, including Africa, the risk of campylobacteriosis is higher due to inadequate production and processing practices, poor water supply and poor sanitary conditions. Campylobacteriosis is aggravated due to lack of monitoring, treatment facilities and health services in developing countries (Kunadu et al., 2020; Phosa et al., 2022). Food quality analyzes have shown that with heat treatment it is possible to inactivate pathogenic microorganisms during chicken cooking. Furthermore, some pathogens or their toxins can survive and cause illness after meals are consumed (Tahreem Khalid et al., 2020). However, the cooking method must be suitable, allowing access to the surface of the chicken and the core or epicenter of the product.

BEST PRACTICES IN POULTRY PRODUCTION

Applying good practices during primary production is the key to ensuring safe food. Therefore, meat hygiene programs are traditionally based on good hygiene practices, these provide a basic food control program, using standard operating practices that illustrate specific steps in the food processing chain (Oloo, 2017). Good hygiene practices at primary production level should involve poultry health and hygiene, records of treatments, feed and food ingredients and relevant environmental factors (Commission, 2005). The production and distribution of chickens must be monitored through poultry identification practices, to allow the origin of the meat to be traced from the slaughterhouse or market to the poultry farm to facilitate the investigation in case of contaminations (Otwey and Kunadu, 2022).

For the welfare of the chickens, they must be kept in properly prepared litter, where the ambient temperature and ventilation rate can be controlled. At the end of each rearing period, all chicken must be removed from the shed to allow cleaning, disinfection as a way of maintaining hygiene and control of pathogenic microorganisms (Muchangos, 2012). Efficient cleaning and disinfection must be accompanied by a high standard of biosecurity, immunization, food and water hygiene, feed additives and bird health monitoring must be in place (Oloo, 2017).

According (Commission, 2005), to ensure the quality and safety of the final product, post-mortem inspection must be carried out in routine screening of carcasses and other relevant parts by methods other than organoleptic inspection (such as microbiological analysis) for suspected hazards. In production there is a need to maintain hygienic practices and environmental sanitation during production to avoid cross-contamination and improve the intestinal health of the poultry (Mak et al., 2022).

According to Ramtahal et al. (2022) for the safe production of chickens, infrastructure must be created to protect the poultry from different climatic conditions, these must be well-sealed environments to limit the entry of other animals and visitor access must be controlled, facilities must also be created adequate storage of animal feed and manure, associated with correct disposal practices for dead poultry waste and other agricultural waste generated. On farms, areas should be designated for the separation of new chicks, sick chicken, healthy flocks and other reared animals, and adequate ventilation and temperature control systems should be provided.

In addition Oloo (2017) suggests that farmers observe best feeding practices and adhere to treatment regimens and antibiotic withdrawal periods used for poultry. The study also emphasizes the collaboration with extension workers, in demonstrating the importance of hygiene for maintaining a healthy chicken intestine, ensuring the safety of the final product. The withdrawal period is defined as the time required for 99.0% of poultry treated according to label instructions to be free of antibiotic residues above the tolerant level (Akinwumi et al., 2017). Oloo (2017) Olutumise et al. (2023) reported the use of good production practices by farmers after acquiring poultry-based skills and education. In addition, the use of good production practices had effects on farmer's income.

The results of observing the implementation of good practices after training showed that 94.2% of farmers adopted adequate vaccination; 92.5% adopted players from a reliable source; 90.0% adopted ideal pre-placement preparation; 86.7% adopted equipment for cleaning, disinfecting and fumigating their homes; 84.2% adopted the timely treatment and removal of dead chicken; 83.3% adopted adequate food management; 81.7% adopted the administration of multivitamins; 79.2% adopted adequate stocking density of chicken; 78.3% adopted ideal feeders/drinkers; 77.5% adopted adequate record keeping; 73.3% adopted adequate medication; 72.5% adopted adequate lighting, heat and humidity; and 71.7% adopted pest and disease vector screening (Olutumise et al., 2023).

Akinwumi et al. (2017) demonstrated that when the labeled dosage and withdrawal period are met, the chicken does not lose weight and also after laboratory tests, no residues of antibiotics were found in the chicken breast muscle. However, many studies discourage the incorporation of antibiotic additives into feeds as a way to prevent microbial contamination, as this can be a likely source of resistance to common antibiotics (Malong, 2022).

According to Mudenda et al. (2022) a national strategy should be developed at the government level to reduce the threat of AMR in animal production and agriculture, which considers the prudent use of antimicrobial agents, investments in disease diagnosis and the detection of antimicrobial residues, the elaboration of biosafety guidelines that fit into intensive production systems and control of antimicrobial residues. Additionally, there is need for pharmaceutical and veterinary professionals to work collaboratively with smallholders in developing strategies to reduce unnecessary use of antibiotics to prevent antimicrobial resistance in the poultry sector (Mdegela et al., 2021).

Additionally Olutumise et al. (2023) also suggests basic education and training through workshops, seminars and extension services because they contribute to the adoption of good production practices. Governments must boost the sector by providing formal land titling that will make poultry farmers feel secure in using technologies and also in building infrastructure that can promote the use of good long-term practices.

PROCESSING METHOD

Poultry meat is a nutritious food, with favorable conditions for the development of microorganisms and has been associated with the presence of foodborne pathogens, such as *E. coli*, *S. aureus*, *Salmonella* and *Campylobacter*, especially when processing conditions are not adequate hygienic. The method of processing chickens in underdeveloped countries, in informal places such as markets and small slaughterhouses, is manual. In most cases, chickens are transported from the production area to the processing site by car, bicycle, hand truck or manually. At the place of slaughter, they are received in the reception area by the sellers, here the chickens are inspected by the sellers and then by the veterinarian (in many cases this does not happen) to check their general health and any manifestations of pests (Oloo, 2017; Otwey and Kunadu, 2022).

After condition checks, chickens are kept in cages with

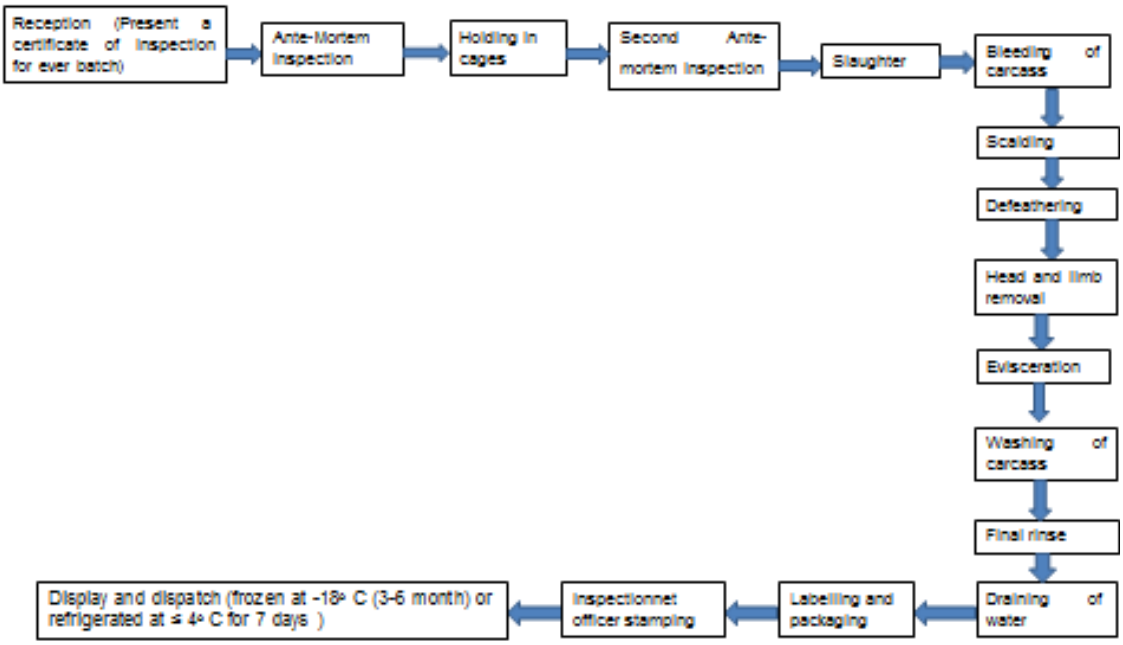

Figure 1. Chicken processing flowchart in informal markets.

a capacity of around 10 birds each, although these cages are often overcrowded due to their limited capacity. The chickens remain in the cages until slaughter and preparation, according to customer needs. Processing takes place according to the flow diagram below adapted from (Oloo, 2017). According to Oloo (2017), chicken processing must be monitored with Hazard Analysis and Critical Control Point (HACCP) to ensure food safety. The author indicates 4 critical control points (CCP): reception of chicken is considered CCP1; at this stage antibiotic residues in chickens must be controlled through laboratory analyses (Figure 1).

CCP2 is the final rinse of the chicken, at this stage, cross-contamination through contact surfaces and water must be controlled, as well as treating the water with chlorine to reduce microorganisms. CCP3 is the packaging of chicken, at this stage it is necessary to monitor toxic residues from the packaging and CCP4 is the conservation of processed chicken in the refrigerator or freezer, at this stage, the temperature must be monitored using thermometers to ensure that the equipment is working properly.

Best practices in poultry processing

Poultry contamination must be controlled during handling, slaughter and processing to ensure the quality and safety of the final product. The hygienic quality of poultry processing surfaces, process water, rinse water and personnel are critical to preventing cross-contamination as a means of protecting consumers from infection and disease (Birgen et al., 2022; Oloo, 2017). Hand and foot cleaning sanitation facilities are important for enhancing staff hygiene and limiting the circulation of pathogens at the processing site. According to Assefa et al. (2023), safe chicken slaughter requires a standard shed with stable power, adequate air flow, clean water, toilets, detergents and freezers. Equipment such as knives, tables and plates made of high-quality, easy-to-clean materials are required and hygienic maintenance is critical to ensure chicken safety.

Hand washing, equipment with appropriate disinfectant during and after activities, a clean work environment, such as table and clean surfaces can favor the reduction of the microbial load, including pathogens such as *Campylobacter* and *Salmonella* (Assefa et al., 2023; Ogutu et al., 2019). Assefa et al. (2023) reported the adoption of good practices among chicken processors in which 34% of the workers answered that they do not go to work when they are sick; this practice limits the contamination of food with pathogenic agents.

According to Nkosi et al. (2021) detergents that present a low residual risk to public health can be used in the processing line, such as sprays of lactic acid, acetic acid and trisodium phosphate, which should be used to reduce most surface pathogens, utensils and meat.

After slaughter the scalding temperature should be monitored to be optimal (52 to 53°C) to allow the reduction of mesophilic aerobic bacteria. In addition, installing a countercurrent system when scalding helps to ensure that the carcasses are in contact with the cleanest water as it exits the scalding tank and will decrease bacterial buildup. Regularly changing contaminated

scalding water and simultaneously introducing fresh water will also remove contaminants from the scald tank.

After plucking, evisceration and washing, the carcass must undergo rapid chilling to a temperature of -6°C to reduce microbial growth. Immediate cooling using water in an adequately controlled system, after washing; retarded the deterioration and development of pathogenic bacteria. The deep bone temperature of the carcass exiting the refrigerator must be less than 7°C; this process can reduce both aerobic plaque counts and coliform bacteria counts by 50% to 90% (Ogutu et al., 2019). Again, it is recommended that efforts are made to avoid bacterial buildup during processing, such as frequent and thorough washing and spray-rinsing, particularly after opening the carcass, after exposing the intestines and after removing the intestines during processing evisceration, will reduce the number of bacterial contaminants. Additionally, frequent rinsing of the plucking machine with clean water and a disinfectant will also reduce bacterial buildup during plucking.

According to Sumbana et al. (2022) refrigeration is a method used to control the growth and multiplication of spoilage bacteria in perishable foods and good storage procedures must be implemented to guarantee the shelf life of the meat and safe food for the consumer. Furthermore, contamination of the carcass can be avoided by using good evisceration techniques and in the event that a carcass has signs of faeces, it must be removed from the line and thoroughly washed, if necessary, with a chlorine solution. Again Khalid et al. (2020) confirms that chlorination can also reduce microbial contamination of work surfaces and equipment in the processing plant, contributing to the cleaning and disinfection of the plant at the end of the working day.

Storing chicken using the freezing system limits the growth of bacteria including pathogenic ones like *Salmonella* and *Campylobacter*. Most studies report that *Salmonella* grows when the temperature is above 5.2^{o} C while thermotolerant *Campylobacter* does not grow below 30^{o} C, freezing reduces the level of contamination of poultry carcasses by inhibiting bacterial growth (Tahreem Khalid et al., 2020). In addition Assefa et al. (2023) advises that training schemes should be adopted for processors on good food handling practices by raising awareness, because wrong practices can be reversed with simple educational campaigns, consumers should also be made aware of the dangers of consuming prepared foods in an unhygienic way, in order to search for safe food.

According to Commission (2005), to ensure the quality and safety of the final product, post-mortem inspection must be carried out in routine screening of carcasses and other relevant parts by methods other than inspection through microbiological analyzes for suspected hazards.

For developing countries, such as Africa, for monitoring chicken quality, techniques for microbiological analysis of more accessible and disposable feeds, ready to use, suitable for food laboratories with few resources can be used. For this, 3M™ Petrifilm™ kits can be used, they are cheap and accessible, they can help in the analysis of quality indicator microorganisms such as mesophilic aerobic bacteria, fecal coliforms and *E. coli*, the presence of these microorganisms allows the presumption of the existence of pathogens that cause foodborne illness.

ALTERNATIVES TO MICROBIOLOGICAL CONTAMINATION IN THE POULTRY PROCESSING CHAIN

Good hygiene practices to ensure safe food with acceptable levels of microorganisms must begin in the primary production of chicken, the shed for raising chicken must be kept hygienic, this includes changing bedding for each production cycle, cleaning the drinkers and feeders, the food must be placed in a place free of animals that could contaminate the food. During production and transport to the slaughterhouse or market, attention must be paid to the welfare of the poultry to prevent them becoming stressed, which can increase the risk of *Salmonella* and *Campylobacter* contamination (Birgen et al., 2022).

Poultry contamination must be avoided during handling, slaughter and processing to protect the public from infection and disease (Oloo, 2017). The author reported the presence of microorganisms (*Bifidobacterium*, *Provetella*) beneficial to the healthy intestine of the chicken, these can be used to promote antagonism with pathogenic bacteria, and this can guarantee the welfare of the animals.

According to Birgen et al. (2022), the poultry must be transported in cages with ample space and in a short period of time to prevent the accumulation of excrement. During unloading, poultry must be prevented from flapping their wings, which can result in considerable dust and micro-organism dispersion through individual picking of each chicken. In markets and slaughterhouses, the infrastructure must be remodeled to prevent the spread of microorganisms during processing. However, there must be a separation of the work places into: delivery area and retention corral, slaughter and bleeding area, processing area and inspection area (remove feathers, heads, paws and viscera), disposal area (intestinal waste, blood, feathers, dirty water and condemned material), packaging and shipping and storage (cold chain) (Assefa et al., 2023).

In slaughterhouses, to limit the contamination and spread of microorganisms, chlorine water spray is used in the evisceration machines and the carcasses are sprayed with chlorinated water (Sumbana et al., 2022). However, in markets where the slaughter process is informal, there is no standard procedure to guarantee the quality of chickens. According to Oloo (2017); Sumbana et al. (2022), a small slaughterhouse could be placed in the

markets with clean and washable walls and tables, protected against flies, where freshly slaughtered poultry are brought daily and kept fresh in a refrigerator or in freezer boxes, isolated with sealed ice packs, this care could ensure hygiene in the processing chain and food safety.

According to Oloo (2017), when the counts of mesophilic aerobic bacteria and fecal coliforms are high, they indicate contamination. In addition to representing adequate hygienic practices and demonstrate that the installation requires the implementation of good handling practices, as well as a safety management system such as HACCP to ensure consumer safety. In addition, the hygienic quality of poultry processing surfaces, process water, rinsing water and personnel are essential to prevent cross-contamination that can lead to adverse effects on the health of consumers. Food contact surfaces must be made of stainless steel to facilitate proper cleaning, sanitization and disinfection and in case of cracks and dents they must be completely covered.

The disposal of solid waste and contaminated water must be monitored to avoid environmental contamination. Both markets and slaughterhouses must be under veterinary supervision, monitor sanitary conditions along the processing chain (Assefa et al., 2023; Oloo, 2017). Several studies find the 3M Petrifilm System ideal for monitoring food safety and microbiological quality in resource-poor developing countries.

Furthermore, the system does not require sophisticated laboratory facilities and can be managed at low cost, as it only requires a room with waterproof and washable walls and floors. Laboratory tables can be small and easily made from solid glass or stainless steel as Petri films are small and therefore cost less to assemble. The necessary laboratory scale, stomacher bag, Bunsen burner and incubator are very accessible. Glassware costs can also be reduced by using disposable pipettes.

According to Dione et al. (2022), in order to improve sanitation, improvements must be made to the slaughter areas, such as training processors at a technical level, guaranteeing the supply of clean water, improving latrines and/or bathrooms, guaranteeing a good system for managing slaughter residues, increasing raising awareness of the importance of a cold chain and improving general hygiene at points of sale. Organic acids are used in the poultry industry as decontaminants, acting to inhibit food spoilage bacteria by reducing the pH lactic acid (1% concentration) retarded microbial growth and increased chicken shelf life during refrigerated storage, S.aureus counts were reduced to 1.2 log CFU/g and mesophilic aerobic bacteria counts were reduced to 1 log and 2 log CFU/g, the count of psychotropic bacteria and *Pseudomonas* the reduction was 0.3 and 0.5 log and the counts of *Enterobacteriaceae*, reduced to 0.4 and 0.7 log CFU/g with application of 0.2 and 1% lactic acid (Ben Braïek and Smaoui, 2021). The authors report that these results are important to ensure chicken quality and increase feed availability through increased shelf life.

Additionally, to avoid cross-contamination during kitchen preparation of chicken, defrosting must be at a temperature of 15^{o}C, secondary ingredients must be prepared before raw meat or never reuse a dirty cutting board for both raw meat and cooked and complete cooking can inactivate *Salmonella* spp., at 70^{o}C for at least 1 min and *Campylobacter* spp., thermotolerant at temperatures above 60^{o}C for more than 1 min (Tahreem Khalid et al., 2020). In addition, chemical decontamination of poultry carcasses, which includes washing with organic acids, chlorinated or electrolyzed water, can be an alternative against microbial contamination.

ALTERNATIVES TO ANTIBIOTICS IN POULTRY PRODUCTION

Antimicrobial resistance is a concern for both developed and developing countries because it affects public and animal health exacerbated by the use of the same antimicrobials in humans and animals (Mudenda et al., 2022). However, antimicrobial resistance is driven by the indiscriminate use of veterinary antibiotics in poultry production, which requires multisectoral interventions, as well as a review of government strategies, policies and regulations on the use of antimicrobials in poultry production (Azabo et al., 2022; Kimera et al., 2020; Mudenda et al., 2022). According to Mudenda et al. (2022), monitoring of antibiotics involves laboratory analysis, ongoing training in antimicrobial management and surveillance of antimicrobial resistance (AMR) among pharmaceutical and veterinary professionals and farmers, in order to promote the rational use of antibiotics.

On the one hand, pharmaceutical professionals can work as animal health service providers to reduce antimicrobial resistance in chickens by monitoring antibiotic use, using the “Access”, “Visualization” and “Reserve” (AWaRe) antibiotic protocol classification from the World Health Organization (WHO) (Azabo et al., 2022; Kimera et al., 2020; Mudenda et al., 2022). On the other hand, the AWaRe classification tool is crucial in monitoring the rational use of antibiotics, optimizing the use of antibiotics, developing programs to reduce antimicrobial resistance (Sharland et al., 2018). However, the strategy to control antimicrobial residues is still limited in developing countries due to the lack of complex laboratory equipment and the high cost required (Lakew et al., 2022).The authors suggest reducing the number of analytes and using a more economical analytical method that allows the detection of multiple residues using a single instrument, without changing the parameters of each analyte. As an alternative method for the simultaneous determination and confirmation of many antibiotic residues, the method of liquid chromatography with UV detection (LC-UV) is proposed.

However, to ensure food safety, poultry production must be free of antibiotics and requires alternative methods to antibiotics in poultry production (Mak et al., 2022). Several studies recommend the investigation and use of alternatives to antibiotics such as probiotics, prebiotics, herbal remedies, vaccinations, dietary supplements, such as minerals, vitamins, enzymes, organic acids and improvements in poultry production practices (Arsène et al., 2022; Bamidele et al., 2022; Kimera et al., 2020; Mak et al., 2022; Oloo, 2017; Ramtahal et al., 2022). Also methods like heat treatment, activated charcoal, resin and UV irradiation can be used as antibiotic inactivators (Arsène et al., 2022). Bamidele et al. (2022) in the comparative study reported that *E. coli* isolates were higher in smallholders, where antibiotics were used in 27.7% and in farms that used ethnoveterinary medicines the isolates were 14.3%. In another study, the increase of *Lactobacillus* bacteria in the intestine of chicken fed with blueberry pomace, mixed with the feed, was reported, demonstrating the bioactive functionality of flavonoids present in fruit products (Islam et al., 2019).

Therefore, probiotics such as *Lactobacillus* spp., *Enterococcus faecium* and *E. faecalis*, *Streptococcus thermophilus* and *Bacillus subtilis*, are suggested as an alternative to antibiotics through their addition to poultry feed, as these may interfere with the colonization of the intestine by pathogenic bacteria through exclusion competitive (Khan et al., 2022). These microorganisms naturally produce volatile organic and fatty acids and aid digestion by breaking down insoluble fiber and improving nutrient absorption metabolism, as well as lowering gut pH to levels that affect pathogenic bacteria such as *E. coli* and *Salmonella* spp.

In addition Oloo (2017) suggests an alternative to reducing the use of antibiotics by introducing beneficial microorganisms into the gut of chicken such as *Bifidobacterium*, *Provetella*, these can be used to create antagonism with pathogenic bacteria. In poultry farms, the use of foods that increase colonization by healthy microorganisms and the reduction in the use of antibiotics can suppress pathogens and improve food absorption, in a healthier way. In poultry, prebiotics, such as fructooligosaccharides, galactooligosaccharides and mannanoligosaccharides can be used as substrates for *Bifidobacterium* bacteria and other lactic acid bacteria due to their ability to promote beneficial bacteria in the gut by accelerating digestion and inhibiting colonization by pathogenic bacteria (Mak et al., 2022; Ricke, 2018).

According Mak et al. (2022), organic acids are used in poultry as antimicrobials; they contribute to the acidification of the intestines by reducing the pH which inhibits the growth of pathogenic bacteria, reducing contamination and favoring the digestion of nutrients. The study revealed that chicken fed a mixture of essential oils such as basil, cumin, lemon, bay leaf, and oregano, had satisfactory growth and significant weight gain, combined with the antioxidant and antimicrobial properties of the oils. Xylanase and glucanase enzymes, also mixed into poultry feed, facilitate food breakdown by allowing access to amino acids and minerals, and promote nutrient absorption (Selaledi et al., 2020).

Again Mlambo et al. (2022) suggests incorporating seaweed into poultry diets, these can improve feed utilization efficiency, growth performance, chicken health, meat stability and quality, and environmental and consumer health. Seaweed has bioactive compounds, with antiviral, antibacterial, antifungal, antioxidant, anticancer, anti-inflammatory, antiallergic, antithrombotic, neuroprotective, hypocholesterolemic and hypoglycemic properties, which makes these compounds essential in optimizing the intestinal environment, inhibiting oxidative stress and inflammation, enhancing immunity and improving growth performance in poultry.

However, several studies recommend the association of all alternatives to antibiotics to provide the potentialities that exist in each one, in a single product, so it can bring more gains similar to those of antibiotics but without the risk of antimicrobial resistance and preserving public health.

CONCLUSION

The microbiological quality and safety of chicken processed in Africa face enormous challenges due to the indiscriminate use of antibiotics and contamination that occurs during production, along with its repercussions from the processing line. Several studies have reported the occurrence of contamination by pathogenic microorganisms such as Salmonella, E. coli, S. *aureus*, and Campylobacter, implicated in disease outbreaks in poultry and humans. In Africa, the commercial production of broiler chicken is the most developed and is accompanied by demands for the implementation of good practice techniques and other instruments that guarantee food safety and consumer protection. Inadequate biosecurity practices in primary production and poor hygiene practices in formal and informal processing units are the main causes of chicken contamination.

The use of antibiotics for prophylactic purposes and treatment of diseases has increased in poultry farms in recent years in Africa, mainly in intensive production, and smallholders are inexperienced in the use of drugs. The Health Management Practices instrument is suggested to ensure hygiene on poultry farms, combined with the antibiotic use monitoring instrument using the classification of the World Organization antibiotics protocol "Access," "Visualization," and "Reserve" (AWaRe) (WHO) for the rational use of antibiotics. The poultry sector is also challenged to phase out the use of antibiotics and introduce alternatives such as prebiotics, probiotics, and plant extracts as a way to combat antimicrobial resistance.

Poultry processors, particularly those in the informal

sector, lack training in matters of good practices and food safety for chicken processing. African governments should contribute to monitoring poultry activities by providing better conditions, including training for producers and processors in this sector.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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