Original Research Article

Antibiotic Profile of *Staphylococcus aureus* on Table Eggs From Ezrad Farms in Iwo Area of State

ABSTRACT

Surface swabs of the table eggs was carried out using sterile swab sticks. These were inoculated on Mannitol Salt Agar and incubated at 37°C for 24 hours. The isolates obtained were morphologically and biochemically characterized. 62% of the isolates obtained were identified as *Staphylococcus aureus.* 0.5 McFarland standard of each *Staphylococcus aureus* isolate was subjected to antibiotic susceptibility test on Muller Hinton Agar using the disc diffusion method. Antibiotic susceptibility was determined by observing and measuring clear zones in millimetres. The antibiogram pattern of *Staphylococcus aureus* on the surface of table eggs from Ezrad farms located in Iwo, Osun State was investigated. *Staphylococcus aureus* isolates were 100% resistant to Augmentin and Cloxacillin while resistance to Ceftazidine, Erythromycin, Gentamycin, Ofloxacin, Cefuroxime and Ceftriaxone were at 96%, 89%, 86%, 82%, 75% and 57% respectively. This study shows high resistance of *Staphylococcus aureus* isolated from egg shells to antibiotics which could pose a serious health problem.

Keywords: Antibiotic susceptibility, Drug resistance, Microbial infection, Poultry eggs, *Staphylococcus aureus*,

INTRODUCTION

The term poultry generally refers to domestic fowl that are raised for their meat and eggs for food. Examples are: chicken, duck, geese, turkey e.t.c. Poultry farming is one of the most important aspect of farming with chicken and turkey being the most reared. More than 50 billion chickens are raised yearly as a source of food. Chickens raised for eggs are layers while those raised for meat are broilers (World Farming Poultry, 2011).

Battery cages are a housing system used for various animal production methods, but primarily for egg-laying hens. The name arises from the arrangement of rows and columns of identical cages connected together, sharing common divider walls, as in the cells of a battery (Horne *et al.*, 2008). Although the term is usually applied to poultry farming, similar cage systems are used for other animals (Leenstra *et al.*, 2016). Battery cages are the predominant form of housing for hens worldwide (Meseret, 2016).

Eggs and meat gotten from poultry are very important sources of folic acid, proteins and other essential nutrients. Chicken meat is relatively cheaper and more affordable compared to other livestock meat. The nutritional value of eggs can be improved to become a functional food (Sparks, 2006; Windhorts, 2008). Poultry animals are able to adapt to almost all areas in the world, they have a high rate of productivity, generate and reproduce rapidly (Smith, 2001**).**

An egg is an oval body laid by a female animal which consists of an ovum surrounded by layers of membrane and an outer covering which nourishes and protects a developing embryo and its nutrient reserves. The poultry egg consists of a protective egg shell, albumen i.e. egg white and vitellus i.e. egg yolk, contained within various thin membranes. The egg shell is generally discarded although every part of the egg is edible. The whole egg and yolk contain significant amounts of proteins and chlorides and are widely used in cookery (FAO, 2008).

Eggs contain two parts; the white and to one part, yolk by weight. The whole mixed egg contains about 65% water, 12% protein and 11% fat. Virtually all of the fat is in the yolk and

12% solids of egg white are virtually all protein. The yolk is rich in fat-soluble vitamins; A, D, E and K and phospholipids including emulsifier lecithin. Eggs are also a good source of iron (FAO, 2013).

Eggs are a chief source of proteins and provide about 25.17g of proteins per 100g of eggs. Other vitamins and minerals found in eggs include; rectinol, riboflavin, folic acid, calcium and potassium (FAO, 2008). Egg white consists primarily of about 90% water into which is dissolved 10% protein with carbohydrate content less than 1% and no fat. The yolk makes up about 33% of the lipid weight of the egg. It contains all of the aft, slightly less than of the protein and most of the other nutrients including chlorine which is an important nutrient for the development of the brain.

Numerous microorganisms are associated with poultry egg surface within a short time and under certain conditions may penetrate into the eggs and grow to cause spoilage (Smith *et al.,* 2000). *Enterobacter aerogenes, Escherichia coli, Citrobacter freundii, Bacillus cereus, Enteroccocus faecalis, Proteus mirabillis, Staphylococcus aureus, Campylobacter jejeuni, Clostridium perfringes, Listeria monocytogens, Yersinia enterocolitica* and *Salmonella spp* for the bacterial isolates while the fungi isolates were *Mucor sp., Rhizopus sp., Aspergillus sp., Fusarium sp.* and *Penicillium sp*. (Ogboghodo *et al.,* 2016). The presence of these microorganisms might constitute a serious risk to consumers especially when they are not properly washed before cooking. *Staphylococcus aureus* cause food borne diseases and symptoms include nausea, vomiting, severe abdominal pain and bloody diarrhoea.

Staphylococcus aureus is easily the most important species of the Staphylococci. It is found in the environment and is frequently seen as normal flora bacteria in people and 20 to 40 percent of adults have *S. aureus* colonized in the nares. It can also colonize without disease in the armpit area, the perineum, skin fold and the vagina. However, *Staphylococcus aureus* is a major opportunistic pathogen that causes a myriad of diseases in humans.

The microscopic appearance of *Staphylococcus aureus* is round and resembles that of a sphere (cocci). Because of the way the bacteria divide and multiply, it will appear in clusters or tetrads. In Greek, *Staphylococcus* means "clusters of grapes" (Ryan *et al.,* 2004). The use of a common bacteriological stain, the Gram stain, helps to identify *S. aureus.* The organism will appears purple using this staining technique and is called gram-positive. When grown on bacteriological media, *Staphylococcus aureus* appears as a large white to golden colony. The majority of the time the colony of *Staphylococcus aureus* produces a zone of haemolysis surrounding the colony. It is not very fastidious and grows well, either aerobically or under anaerobic conditions and produces good growth within 24 hours (Varrone *et al*., 2014).

Staphylococcus aureus produce a wide variety of virulence factors that allow it to produce many different types of disease. produces various enzymes such as coagulase (bound and free coagulase) which clots plasma and coats the bacteria cell probably to prevent phagocytosis. Hyaluronidase also known as spreading factor breaks down hyaluronic acid and helps in spreading it. *Staphylococcus aureus* also produce deoxyribonuclease which helps to break down DNA, lipase to digest lipids, staphylokinase to dissolve fibrin and aid in spread, and beta-lactamase for drug resistance.

Depending on the strain, *Staphylococcus aureus* is capable of secreting several exotoxins which can be classified into 3 groups many of these toxins are associated with specific diseases (Dingles *et al.,* 2000). Superantigens: they can induce Toxic Shock Syndrome (TSS). This group includes the toxins TSST-1 and enterotoxin type B, which causes TSS associated with tampon use. TSS is characterised by fever, erythematous rash, low blood pressure, shock, multiple organ failure and skin peeling. Lack of antibody to TSST-1 plays a part in the pathogenesis of TSS. Other strains of *S. aureus* can produce an enterotoxin that is the causative agent of a type of gastroenteritis. This form of gastroenteritis is self-limiting,

characterized by vomiting and diarrhoea one to six hours after ingestion of the toxin, with recovery in eight to 24 hours. Symptoms include nausea, vomiting, diarrhoea, and major abdominal pain (Jarraud *et al.,* 2001; Becker *et al.,* 2003).

Exfoliative toxins: They are exotoxins implicated in the disease staphylococcal scalded skin syndrome (SSSS), which occurs most commonly in infants and young children. It also may occur as epidemics in hospital nurseries. The protease activity of the exfoliative toxins causes peeling of the skin observed with SSSS (Berker *et al.,* 2003).

Staphylococcus aureus causes a variety of pus-forming (suppurative) infections and toxinoses in humans. The presence of *Staphylococcus aureus* does not always indicate an infection; *Staphylococcus aureus* can survive for several hours to weeks and months on dry environmental surfaces depending on the strain. It causes superficial skin lesions such as boils, pimples, impetigo and furuncles; more serious infection such as scalded skin syndrome, pneumonia, mastitis, abscesses, meningitis and cellulitis folliculitis and urinary tract infections; and deep-seated infections such as toxic shock syndrome (TSS), osteomyelitis, bacteraemia, and endocarditis (Todar, 2008). *Staphylococcus aureus* is a major cause of hospital acquired infection of surgical wounds and infections associated with medical devices. It also causes food poisoning by releasing enterotoxins into food and TSS by releasing super antigens into the blood stream and is often the cause of postsurgical wound infections. *S. aureus* is a leading cause of bloodstream infections throughout much of the industrialized world (Rasummen *et al.,* 2011). Infection is generally associated with breakages in the skin or mucosal membranes due to surgery, injury, or use of intravascular devices such as catheters, hemodialysis machines, or injected drugs (Tong *et al.,* 2015; Rasmussen *et al.,* 2011). Once the bacteria have entered the bloodstream, they can infect various organs, causing infective endocarditis, septic arthritis, and osteomyelitis (Rasummen *et al.,* 2011). This disease is particularly prevalent and severe in the very young and very old (Tong *et al.,* 2015).

Staphylococcus aureus is more prevalent in atopic dermatitis patients. It is mostly found in fertile, active places such as the armpits, hair and scalp. Larger pimples that appear in those areas may exacerbate the infection if lacerated. This can also lead to staphylococcal scalded skin syndrome (SSSS). A severe form is observed in neonatals (Curren and Al-Sahili, 1980). *Staphylococcus aureus* can survive on dogs, cats, horses and causes bumble foot in chickens. It is also one of the causal agents of mastitis in dairy cows. Its large polysaccharide capsule protects the organism from recognition by the cow's immune defence (Karama *et al.,* 2003).

The emergence of resistance of bacteria to antibiotics is a common phenomenon. Emergence of resistance often reflects evolutionary processes that take place during antibiotic therapy. The antibiotic treatment may select for bacterial strains with physiologically or genetically enhanced capacity to survive high doses of antibiotics. Under certain conditions, it may result in preferential growth of resistant bacteria, while growth of susceptible bacteria is inhibited by the drug (Levy, 1994). Antibiotics such as penicillin and Erythromycin, which used to have a high efficacy against many bacterial species and strains, have become less effective, due to the increased resistance of many bacterial strains (Pearson, 2007).

Resistance may take the form of biodegradation of pharmaceuticals, such as sulfamethazine-degrading soil bacteria introduced to sulfamethazine through medicated pig faeces (Topp *et al.,* 2013). The survival of bacteria often results from an inheritable resistance (Witte, 2004), but the growth of resistance to antibacterials also occurs through horizontal gene transfer. Horizontal transfer is more likely to happen in locations of frequent antibiotic use (Dyer, 2003). Antibacterial resistance may impose a biological cost, thereby reducing fitness of resistant strains, which can limit the spread of antibacterial-resistant bacteria, for example, in the absence of antibacterial compounds. Additional mutations, however, may compensate for this fitness cost and can aid the survival of these bacteria (Adersson, 2006).

Staphylococcus aureus is one of the microorganisms found on the surface of table eggs and can be transferred to humans via handling of poultry eggs. The prevalence of *Staphylococcus aureus* is reduced in developed countries where table eggs are hygienically treated before being released to the general public for use. However, in developing countries eggs are not subjected to hygienic treatments. It is expected that the absence of these treatment would aid in cross contamination of microorganisms from the surface of eggs to humans and to the environment at large. There is a need to have a reliable data on how *S. aureus* is associated with the surface of farm eggs and gather information on the antibiotic profile.

The aim of this research work is to isolate and determine the antibiotic profile of *Staphylococcus aureus* isolated from the surface of day old table eggs from Ezard Iwo, Osun State.

MATERIALS AND METHODS

2.1 Materials

The materials that were used in this research work included crates of eggs, sterile swab sticks, test tubes, test tube rack, conical flasks, cotton wool, sterile Petri-dishes, inoculating loop, spirit lamp, ethanol, weighing balance, measuring cylinder, beaker, Durham tubes, powdered gloves, sterile water, normal saline water and 0.5 McFarland solution.

The growth media used were: Mannitol Salt Agar (MSA), Muller Hinton Agar (MHA) and Nutrient Agar (NA). The reagents used included: methyl red, hydrogen peroxide and Kovac's reagent.

2.2 Sterilization of Materials

The work bench was sterilized using cotton swab soaked in 70% ethanol before and after every use. Inoculating loop was flamed till red hot using spirit lamp before and after every use. All glass wares such as conical flasks, test tubes, beakers, slant bottles e.t.c. and media were sterilized in the autoclave at 121°C for 15 minutes before use.

2.3 Media Preparation and Composition

Laboratory media used for this research were Mannitol Salt Agar (MSA), Nutrient Agar (NA) and Muller Hinton Agar.

2.3.1 Mannitol Salt Agar (MSA)

This is used for selective isolation and differentiation of *Staphylococcus aureus*.

Composition	**g/m**
Sodium chloride	75.0
Protease peptone	10.0
Mannitol	10.0
Beef extract	1.0
Phenol red	0.025
Agar	15.0

pH 7.4 ± 0.2

2.3.2 Nutrient Agar (NA)

This medium is a very common one used in laboratories and is particularly good for making pure cultures on slants and sub-culturing of pure bacterial isolates.

Composition	**g/l**
Peptone	5.0
Meat extracts	1.0
Sodium chloride	2.0
Agar	15.0

Preparation

Nutrient agar was prepared according to the manufacturer's instructions and specification which stated that 28g of the agar powder was dissolved in 100ml of distilled water and sterilized in the autoclave for 15 minutes at 121°C. After sterilization the medium was then

allowed to cool to a temperature of 45° - 47°C, poured into sterile Petri dishes, swirled for even distribution and allowed to gel.

2.3.3 Muller Hinton Agar

This medium is used for antibiotic sensitivity or susceptibility tests.

Composition	**g/l**
Peptone	17.5
Beef infusion solids	2.0
Starch	1.5
Agar	17.0

Preparation

38g of the medium was added into 1 litre of distilled water and mixed homogenously. The preparation was then sterilized in the autoclave at 121°C for 15 minutes. After autoclaving, the medium was allowed to cool to temperature of 45°- 47°C, poured into sterile Petri dishes, swirled for even distribution and allowed to gel.

2.4 Collection of Samples

A crate of eggs containing 30 pieces of a day-old eggs were collected from Ezrad farms, Iwo, Osun State, for three weeks. In all, 90 pieces of day-old eggs were used.

2.4.1 Inoculation of Samples

In the laboratory, microbial sampling was carried out on the eggs. In each crate of egg, a sterile swab stick, moistened in normal sterile saline water, was used to swab the external surface of every two egg-shell and streaked on freshly prepared Mannitol Salt Agar plate. In all, 15 streaked sample plates were prepared from each crate of egg. The plates were incubated at 37°C for 18 – 24 hours. Afterwards, yellow colony growths obtained were presumed *Staphylococcus aureus* and recorded. The presumed colonies were purified by repeated re-streaking on fresh Mannitol Salt Agar plates until pure colonies were obtained. One pure isolate colony from each sample plate was stored in a sterile agar nutrient agar slant and kept in a refrigerator until when needed.

2.5 Identification of Isolate

Each presumed S. aureus colony isolate was characterised based on standard microbial identification procedures such as colony morphology, Gram stain reaction, fermentation of sugars, methyl red reaction, citrate test and motility test.

2.5.1 Gram Staining

Standard Gram staining procedure were carried out on the pure isolate obtained as described by Fawole and Oso (2001).

A smear of the organism was prepared by placing a small drop of sterile water on a sterile slide and a loopful of an 18 hours old culture was taken using a sterile inoculating loop and rubbed on the drop of sterile to form a thin smear. The smear was heat fixed by carefully passing over a flame. The smear was the flooded with a drop of crystal violet stain for 30 -60 seconds then rinsed off gently in running water. One drop of Gram's iodine which served as a mordant was added to the smear and allowed to stand for 60 seconds and rinsed off gently with water. Small drops of 70% alcohol was placed on the smear (which served as a decolorizing agent) and gently rinsed off. Safranin red was added to the smear to counter stain and it was allowed to stand for 1 minute after which it was gently rinsed off. The smear was the air dried and a drop of immersion oil was added.

A microscopic examination was carried out under an oil immersion objective lens using a magnification strength of X100. A purple colouration indicated Gram positive bacteria, while a red or pink colouration indicated a Gram negative bacteria.

2.5.2 Catalase Test

The principle of this test is to detect the activity of the enzyme catalyse which leads to the breakdown of hydrogen peroxide to give oxygen and water. One drop of 3% hydrogen

peroxide was placed on a clean, grease free slide. Using an inoculating loop, a pure bacterial colony was picked and placed on the slide containing the hydrogen peroxide and mixed together. Bubble formation was observed which indicates the presence of the enzyme catalase while no formation of the bubbles indicates the absence of the enzyme catalase (Brown, 2005).

2.5.3 Motility Test

This test is carried out to find out if the isolated organism is a motile organism or a non-motile organism. A pure bacteria colony was picked using a sterilized inoculating pin or needle and gently stabbed into a test tube containing a sterile semi-solid nutrient agar medium. The test tube was then incubated at 37°C for 24 hours. After this, motility was observed as a spiral growth from the point of inoculation to the bottom of the test tube due to the migration and movement of motile bacteria (Brown, 2005).

2.5.4 Methyl Red Test (MR)

Composition of MR broth	**g/l**
Dextrose	0.5g
KH_2PO_4	0.5g
Peptone	0.5g
Methyl red powder	0.1g
Distilled water	100ml

Procedure:

Five millilitres of the broth was dispensed into test tubes plugged with sterile cotton wool and sterilized in the autoclave at 121°C for 15 minutes. After autoclaving, it was allowed to cool down and the bacterial organism was inoculated into the test tubes. It was then incubated at 37° for 3 days. After incubation, few drops of methyl red was added to and observed for colour changes. A red colouration indicated a positive reaction (Arora and Arora, 2007).

2.5.5 Voges Proskaeur Test (VP)

Composition	**g/l**
Dextrose	0.5g
KH_2PO_4	0.5g
Peptone	0.5g
Distilled water	100ml

Composition of Reagent

Barrit's ethanolic solution of α-naphtol and 40% potassium hydroxide (KOH).

Procedure:

Five millilitres of the broth was dispensed into test tubes and plugged with cotton wool. It was then sterilized in the autoclave at 121°C for 15 minutes. After autoclaving, it was allowed to cool and the organism was inoculated into the test tubes and incubated at 37°C for 3 days (72 hours). After incubation, 5% α-naphtol solution and 40% potassium hydroxide was added to the culture and shaken, it was then observed for colour change. The formation of a red colour indicated a positive reaction (Tiwari *et al.*, 2009).

2.5.6 Indole Test

Composition	**g/l**
Tryptone water	0.5g
Sodium chloride	0.5g
Distilled water	100ml

Test Reagent: Kovac's Reagent

Procedure:

Five millilitres of the prepared solution was dispensed into test tubes, plugged with cotton wool and sterilized in the autoclave at 121°C for 15 minutes. After autoclaving, it was allowed to cool down and the organism was inoculated into the test tubes and incubated at

37°C for 3 days. After incubation, Kovac's reagent was added into the culture, mixed thoroughly, allowed to settle and observed for colour change. The formation of a red coloured ring at the top indicated a positive reaction while no colour change indicated a negative reaction (PHE, 2014).

2.5.7 Citrate Utilization Test

Sterile Simmons citrate agar was prepared, mixed with sterile water and stirred using a stirrer and hot plate. Five millilitres (5ml) of the solution was dispensed into test tubes, plugged with cotton wool and sterilized in the autoclave for 15 minutes at 121°C. After autoclaving, it was allowed to cool down and the organism was inoculated into the test tubes and incubated at 37°C for 2 – 3 days (48 – 72 hours). A colour change from green to blue indicated a positive reaction while no colour change indicated a negative reaction (Tiwari *et al.*, 2009).

2.5.8 Sugar Fermentation Tests

This test is carried out to determine the ability of an organism to ferment sugars. The sugars tested for include; glucose, lactose, sucrose and mannitol. Peptone solution of each of the sugars was used in ratio of 3:1 and 2ml of 0.01% phenol red was dissolved in 100ml of distilled water. Into each test tube 5ml of the solution was dispersed and Durham tube was inserted into each of the test tubes making sure there was no bubble. It was then inoculated with the bacterial isolates. The test tubes were incubated at 37° C for 72 hours. A change in colour of the medium indicated the production of acid. A displacement of the solution in Durham tube by air (carbon dioxide) indicated the production of gas (Arora and Arora, 2007).

2.5.9 Starch Hydrolysis

Nutrient agar and 1% soluble starch was mixed and sterilized by autoclaving. It was poured, allowed to gel and the test organism was inoculated and incubated for 48 hours. After incubation, iodine was poured on the region where growth was obtained. A positive result showed a clear zone around the area because starch had been hydrolysed. No clear zone after addition of iodine indicates a negative result (Brown, 2005).

2.6 Antibiotic Sensitivity Test

For antimicrobial sensitive test Muller Hinton agar is used. It was prepared according to the manufacturer's instructions. The agar was the sterilized by autoclaving at 121°C for 15 minutes. After autoclaving it was allowed to cool and the poured into sterile Petri dishes and gently swirled for even distribution before allowing it to gel. Each test tube to be used was sterilized by cleaning the inside with ethanol and flaming the tip. Two ml (2ml) of normal saline water was dispensed into the sterile test tubes and a loopfull of the organism was inoculated into the test tubes containing the normal saline. The turbidity of the organism in the test tube was then visually compared to 0.5 Mc Farland's standard then streaked all over the Muller Hinton plate using an inoculating loop. Gram positive sensitivity discs were then carefully placed on each plates using sterile forceps and incubated at 37°C for 24 hours.

A clear zone without microbial around the antibiotic indicated susceptibility while a non-clear zone with microbial growth indicates resistivity of the organism to the antibiotic.

RESULTS

3.1 Identification and Incidence of Obtained Isolates

A total of 45 samples swabs of the surface of table eggs were collected. Twenty-eight presumed *Staphylococcus aureus* isolates were obtained and they were morphologically and biochemically identified as *Staphylococcus aureus* (Table 1).

3.2 Antibiotic pattern of *Staphylococcus aureus* from table eggs

For each of the weeks in which this research was carried out *Staphylococcus aureus* showed 100% resistance to Augmentin and Cloxacillin antibiotics. Ceftazidime, Erythromycin and Cefuroxime also showed a high level of resistance with 90%, 80% and 70% respectively. In the second week of research, *Staphylococcus aureus* showed 100% resistance to Ceftazidime,

Augmentin, Ofloxacin, Cloxacillin and Gentamicin. Erythromycin also recorded a high level of resistance at 86%.

In the third week of work, *Staphylococcus aureus* was 100% resistant to Ceftazidime, Augmentin, Cloxacillin and Erythromycin with high resistivity of Cefuroxime, Ofloxacin and Gentamycin at 91%, 91% and 82% respectively as seen in Table 2. Overall antibiogram profile of *Staphylococcus aureus* showed Augmentin and Cloxacillin having the highest level of resistance at 100% resistivity. Ceftazidime was also highly resistant at 96%. The antibiotic which *Staphylococcus aureus* showed the highest susceptibility to is Ofloxacin with 18% as shown in Table 3.

Table 4 shows the multi-drug resistant pattern of isolated *Staphylococcus aureus*. 36% of the *Staphylococcus aureus* isolates were resistant to the combination of Ceftazidime, Ceftriaxone, Cefuroxime, Augmentin, Ofloxacin, Cloxacillin, Erythromycin and Gentamicin.

Table 1: Morphological and biochemical characteristics of isolated organisms.

	G.S	Sha	Cat	Mot	MR	VP	Ind	Cit	Sta	Glu	Lac	Man	Suc	P.O
1	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
2	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
3	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
4	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
5	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
6	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
7	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
8	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
9	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
10	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
11	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
12	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
13	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
14	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
15	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
16	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
17	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
18	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
19	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
20	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
21	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
22	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
23	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
24	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
25	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
26	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
27	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*
28	+	C	+	-	+	+	-	+	+	+	+	+	+	*S. a*

Key: + : positive, - : negative, C: cocci, G.S: Gram stain, Sha: shape, Cat: catalase, Mot: motility, MR: methyl red, VP: Voges Proskaeur, Ind: indole, Cit: citrate, Sta: starch hydrolysis, Glu: glucose, Lac: lactose, Man: mannitol, Suc: sucrose, P.O: probable organism, *S. a*: *Staphylococcus aureus*.

Table 4 shows the multi-drug resistant pattern of isolated *Staphylococcus aureus*. 36% of the *Staphylococcus aureus* isolates were resistant to the combination of Ceftazidime, Ceftriaxone, Cefuroxime, Augmentin, Ofloxacin, Cloxacillin, Erythromycin and Gentamicin.

Table 2: Antibiotic susceptibility of *Staphylococcus aureus* isolated from egg surface

	WEEK ONE N = 11			**WEEK TWO N = 06**			**WEEK THREE N = 11**		
	S%	I%	R%	S%	I%	R%	S%	I%	R%
CAZ	10	0	90	0	0	100	0	0	100
CTR	10	40	50	0	43	57	18	18	64
CRX	20	10	70	14	29	57	9	0	91
AUG	0	0	100	0	0	100	0	0	100
OFL	40	0	60	0	0	100	9	0	91
CXC	0	0	100	0	0	100	0	0	100
ERY	20	0	80	0	14	86	0	0	100
GEN	20	0	18	0	0	100	18	0	82

Key: CAZ: Ceftazidime, CTR: Ceftriaxone, CRX: Cefuroxime, AUG: Augmentin, OFL: Ofloxacin, CXC: Cloxacillin, ERY: Erythromycin, GEN: Gentamicin, S: susceptible, I: intermediate and R: resistant.

Table 3: Overall Antibiotic Profile of *Staphylococcus aureus* on table eggs surface

ANTIBIOTICS	**S%**	**I%**	**R%**
CAZ	4	0	96
CTR	11	32	57
CRX	14	11	75
AUG	0	0	100
OFL	18	0	82
CXC	0	0	100
ERY	7	4	89
GEN	14	0	86

Key: CAZ: Ceftazidime, CTR: Ceftriaxone, CRX: Cefuroxime, AUG: Augmentin, OFL: Ofloxacin, CXC: Cloxacillin, ERY: Erythromycin, GEN: Gentamicin, S: susceptible, I: intermediate, R: resistant.

Table 4: Multi-drug resistance of *Staphylococcus aureus* isolated from Ezrad Farms Iwo

ANTIBIOTICS	OCCURANCE	PERCENTAGE
CAZ CTR AUG OFL CXC ERY GEN	3	11
CTR CRX AUG CXC ERY GEN	1	3.5
CAZ CTR CRX CXC ERY	1	3.5
CAZ CTR CRX AUG OFL CXC ERY GEN	10	36
CAZ CRX AUG OFL CXC ERY GEN	5	18
CAZ CRX AUG CXC	1	3.5
CAZ CRX AUG OFL CXC ERY GEN	1	3.5
CAZ CRX AUG CXC GEN	1	3.5
CAZ CTR AUG OFL CXC GEN	1	3.5
CAZ AUG OFL CXC ERY GEN	2	7
CAZ CRX AUG OFL CXC ERY	1	3.5
CAZ CRX AUG CXC ERY	1	3.5

Key: CAZ: Ceftazidime, CTR: Ceftriaxone, CRX: Cefuroxime, AUG: Augmentin, OFL: Ofloxacin, CXC: Cloxacillin, ERY: Erythromycin, GEN: Gentamicin.

18% of the obtained *Staphylococcus aureus* were also resistant to Ceftazidime, Cefuroxime, Augmentin, Ofloxacin, Cloxacillin, Erythromycin and Gentamicin. The susceptible and resistant pattern of the antibiotics to *Staphylococcus aureus* according to the classes they belong to is represented in Figure 1.

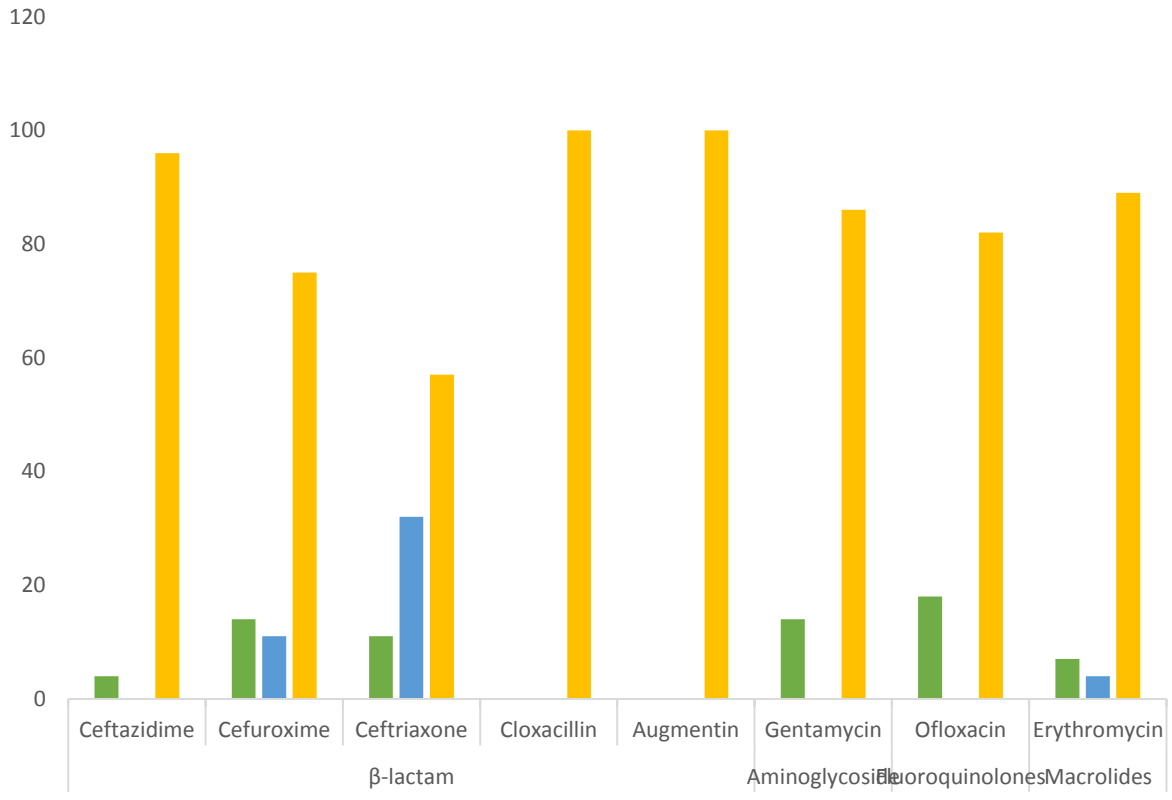

Fig 1: Antibiotic Profile of the Different classes of Antibiotics Used

DISCUSSION AND CONCLUSION

In this study, an incidence of 62.2% *Staphylococcus aureus* was observed on the surfaces of egg shells which is similar to 58.9% reported by Stepien-Pysniak *et al.,* (2009) on surface eggs in Egypt. An incident rate of 100% was also observed by Jehan *et al.,*(2014) on surface eggs in Egypt. This implies that *S. aureus* are more or less frequently found on egg shell surfaces. Sources of *S. aureus* contamination may range from the poultry bird itself, the environment as well as poultry egg handlers and the hygiene practise. The poultry system practised may also serve as a source of horizontal transmission of the organism. Furthermore, *Staphylococcus aureus* on the surface of egg shells are potential microbial source of contamination to the egg content. Wissman (2006) has reported that an embryo can die within 48 hours of exposure to *Staphylococcus aureus.* The presence of *S. aureus* on human skin can also cause cross-contamination and transfer from person to person via contact.

The isolated *Staphylococcus aureus* showed 100% resistance to Augmentin and Cloxacillin which is similar to that recorded by Otajevwo and Momoh (2013) in Delta State, Nigeria. *Staphylococcus aureus* was also 89% and 86% resistant to Erythromycin and Gentamycin, respectively which is similar to 75% that was recorded by Jayatilleke and Bandara (2010) in New York. From results obtained, *Staphylococcus aureus* showed high resistance to β-lactam antibiotics such as Ceftazidime, Augmentin and Cloxacillin, implying these antibiotics may not be suitable for treating staphylococci diseases in chickens. Dhand *et al.* (2001) have suggested that β- lactams be used in combination with other antibacterials to improve outcomes in difficult-to-treat infections caused by *S. aureus* on the basis that β-lactam, despite the phenotypic resistance of the organism, has resulted in changes to the bacterial surface promoting enhanced binding and activity of other antibiotics such as daptomycin.

36% of the isolated *Staphylococcus aureus*, in this study, showed multidrug resistance to the combination of the following antibiotics; Ceftazidime, Augmentin, Oflaxcin, Cloxacillin, Erythromycin, Ceftriaxone, Gentamycin and Cefuroxime. 18% of the isolated *Staphylococcus aureus* were also resistant to the combination of Ceftazidime, Cefuroxime, Augmentin, Ofloxacin, Cloxacillin, Erythromycin and Gentamicin. Treatment of infections caused by *S. aureus* is often complicated by the high prevalence of multi-drug resistant strains which are a consequence of the indiscriminate and inappropriate use of antimicrobials associated with vertical and horizontal resistance gene transfer (Hiramastsu *et al.,* 2013). Microorganisms can survive due to the ability to adapt to antimicrobial agents. They do so via spontaneous mutation or by DNA transfer. This process enables bacteria such as *Staphylococcus aureus* to oppose the action of certain antibiotics rendering the antibiotics ineffective (Bennet, 2008). *Staphylococcus aureus* employs several mechanisms such as efflux mechanisms to remove antibiotics and attaining multi-drug resistance (Li and Nikaido, 2009). Antibiotic resistant bacteria are able to transfer copies of DNA that code for a mechanism of resistance to other bacteria including strains that are distantly related to them. The newly resistant strains are also able to pass on the resistant genes and by so doing generations of antibiotics resistant bacteria are produced (Hussain, 2015).

In conclusion, the research carried out showed that there was relatively high incidence of *Staphylococcus aureus* on the surface of table eggs. It is suggested that strict hygienic practices on farms and by egg handlers will help reduce the spread of *Staphylococcus aureus* on egg surfaces. Poultry eggs can be given some measure of hygiene treatment before release to the community thus reducing the spread of possible microorganisms associated with egg shell surface. Antibiotic resistance of *Staphylococcus aureus* in poultry to numerous antibiotics has made it challenging to treat and this may lead to a public health hazard.

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