

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 Ceftazidime, 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

48 12% solids of egg white are virtually all protein. The yolk is rich in fat-soluble vitamins; A,
49 D, E and K and phospholipids including emulsifier lecithin. Eggs are also a good source of
50 iron (FAO, 2013).

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

58 Numerous microorganisms are associated with poultry egg surface within a short time and
59 under certain conditions may penetrate into the eggs and grow to cause spoilage (Smith *et al.*,
60 2000). *Enterobacter aerogenes*, *Escherichia coli*, *Citrobacter freundii*, *Bacillus cereus*,
61 *Enterococcus faecalis*, *Proteus mirabilis*, *Staphylococcus aureus*, *Campylobacter jejuni*,
62 *Clostridium perfringens*, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Salmonella spp*
63 for the bacterial isolates while the fungi isolates were *Mucor sp.*, *Rhizopus sp.*, *Aspergillus*
64 *sp.*, *Fusarium sp.* and *Penicillium sp.* (Ogboghodo *et al.*, 2016). The presence of these
65 microorganisms might constitute a serious risk to consumers especially when they are not
66 properly washed before cooking. *Staphylococcus aureus* cause food borne diseases and
67 symptoms include nausea, vomiting, severe abdominal pain and bloody diarrhoea.

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

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

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

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

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

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

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

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

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

139 Resistance may take the form of biodegradation of pharmaceuticals, such as sulfamethazine-
140 degrading soil bacteria introduced to sulfamethazine through medicated pig faeces (Topp *et*
141 *al.*, 2013). The survival of bacteria often results from an inheritable resistance (Witte, 2004),
142 but the growth of resistance to antibacterials also occurs through horizontal gene transfer.
143 Horizontal transfer is more likely to happen in locations of frequent antibiotic use (Dyer,
144 2003). Antibacterial resistance may impose a biological cost, thereby reducing fitness of
145 resistant strains, which can limit the spread of antibacterial-resistant bacteria, for example, in
146 the absence of antibacterial compounds. Additional mutations, however, may compensate for
147 this fitness cost and can aid the survival of these bacteria (Adersson, 2006).

148 *Staphylococcus aureus* is one of the microorganisms found on the surface of table eggs and
149 can be transferred to humans via handling of poultry eggs. The prevalence of *Staphylococcus*
150 *aureus* is reduced in developed countries where table eggs are hygienically treated before
151 being released to the general public for use. However, in developing countries eggs are not
152 subjected to hygienic treatments. It is expected that the absence of these treatment would aid
153 in cross contamination of microorganisms from the surface of eggs to humans and to the
154 environment at large. There is a need to have a reliable data on how *S. aureus* is associated
155 with the surface of farm eggs and gather information on the antibiotic profile.
156 The aim of this research work is to isolate and determine the antibiotic profile of
157 *Staphylococcus aureus* isolated from the surface of day old table eggs from Ezard Iwo, Osun
158 State.

MATERIALS AND METHODS

2.1 Materials

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

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

2.2 Sterilization of Materials

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

2.3 Media Preparation and Composition

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

2.3.1 Mannitol Salt Agar (MSA)

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

Composition

	g/m
179 Sodium chloride	75.0
180 Protease peptone	10.0
181 Mannitol	10.0
182 Beef extract	1.0
183 Phenol red	0.025
184 Agar	15.0
185 pH 7.4 ± 0.2	

2.3.2 Nutrient Agar (NA)

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

Composition

	g/l
190 Peptone	5.0
191 Meat extracts	1.0
192 Sodium chloride	2.0
193 Agar	15.0

Preparation

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

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

200 **2.3.3 Muller Hinton Agar**

201 This medium is used for antibiotic sensitivity or susceptibility tests.

202 **Composition**

	g/l
203 Peptone	17.5
204 Beef infusion solids	2.0
205 Starch	1.5
206 Agar	17.0

207 **Preparation**

208 38g of the medium was added into 1 litre of distilled water and mixed homogenously. The
209 preparation was then sterilized in the autoclave at 121°C for 15 minutes. After autoclaving,
210 the medium was allowed to cool to temperature of 45°- 47°C, poured into sterile Petri dishes,
211 swirled for even distribution and allowed to gel.

212 **2.4 Collection of Samples**

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

215 **2.4.1 Inoculation of Samples**

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

225 **2.5 Identification of Isolate**

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

229 **2.5.1 Gram Staining**

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

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

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

245 **2.5.2 Catalase Test**

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

248 peroxide was placed on a clean, grease free slide. Using an inoculating loop, a pure bacterial
249 colony was picked and placed on the slide containing the hydrogen peroxide and mixed
250 together. Bubble formation was observed which indicates the presence of the enzyme catalase
251 while no formation of the bubbles indicates the absence of the enzyme catalase (Brown,
252 2005).

253 **2.5.3 Motility Test**

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

260 **2.5.4 Methyl Red Test (MR)**

261 **Composition of MR broth**

	g/l
262 Dextrose	0.5g
263 KH ₂ PO ₄	0.5g
264 Peptone	0.5g
265 Methyl red powder	0.1g
266 Distilled water	100ml

267 **Procedure:**

268 Five millilitres of the broth was dispensed into test tubes plugged with sterile cotton wool and
269 sterilized in the autoclave at 121°C for 15 minutes. After autoclaving, it was allowed to cool
270 down and the bacterial organism was inoculated into the test tubes. It was then incubated at
271 37° for 3 days. After incubation, few drops of methyl red was added to and observed for
272 colour changes. A red colouration indicated a positive reaction (Arora and Arora, 2007).

273 **2.5.5 Voges Proskauer Test (VP)**

274 **Composition**

	g/l
275 Dextrose	0.5g
276 KH ₂ PO ₄	0.5g
277 Peptone	0.5g
278 Distilled water	100ml

279 **Composition of Reagent**

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

281 **Procedure:**

282 Five millilitres of the broth was dispensed into test tubes and plugged with cotton wool. It
283 was then sterilized in the autoclave at 121°C for 15 minutes. After autoclaving, it was
284 allowed to cool and the organism was inoculated into the test tubes and incubated at 37°C for
285 3 days (72 hours). After incubation, 5% α -naphthol solution and 40% potassium hydroxide
286 was added to the culture and shaken, it was then observed for colour change. The formation
287 of a red colour indicated a positive reaction (Tiwari *et al.*, 2009).

288 **2.5.6 Indole Test**

289 **Composition**

	g/l
290 Tryptone water	0.5g
291 Sodium chloride	0.5g
292 Distilled water	100ml

293 **Test Reagent: Kovac's Reagent**

294 **Procedure:**

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

298 37°C for 3 days. After incubation, Kovac's reagent was added into the culture, mixed
299 thoroughly, allowed to settle and observed for colour change. The formation of a red coloured
300 ring at the top indicated a positive reaction while no colour change indicated a negative
301 reaction (PHE, 2014).

302 **2.5.7 Citrate Utilization Test**

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

310 **2.5.8 Sugar Fermentation Tests**

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

319 **2.5.9 Starch Hydrolysis**

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

325 **2.6 Antibiotic Sensitivity Test**

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

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

338 **RESULTS**

339 **3.1 Identification and Incidence of Obtained Isolates**

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

343 **3.2 Antibiotic pattern of *Staphylococcus aureus* from table eggs**

344 For each of the weeks in which this research was carried out *Staphylococcus aureus* showed
345 100% resistance to Augmentin and Cloxacillin antibiotics. Ceftazidime, Erythromycin and
346 Cefuroxime also showed a high level of resistance with 90%, 80% and 70% respectively. In
347 the second week of research, *Staphylococcus aureus* showed 100% resistance to Ceftazidime,

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

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

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

360 **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	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
2	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
3	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
4	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
5	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
6	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
7	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
8	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
9	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
10	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
11	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
12	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
13	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
14	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
15	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
16	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
17	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
18	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
19	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
20	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
21	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
22	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
23	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
24	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
25	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
26	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
27	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>
28	+	C	+	-	+	+	-	+	+	+	+	+	+	<i>S. a</i>

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

365

366

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

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

	WEEK ONE			WEEK TWO			WEEK THREE		
	N = 11			N = 06			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

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

374

375 **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

376

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

380

381

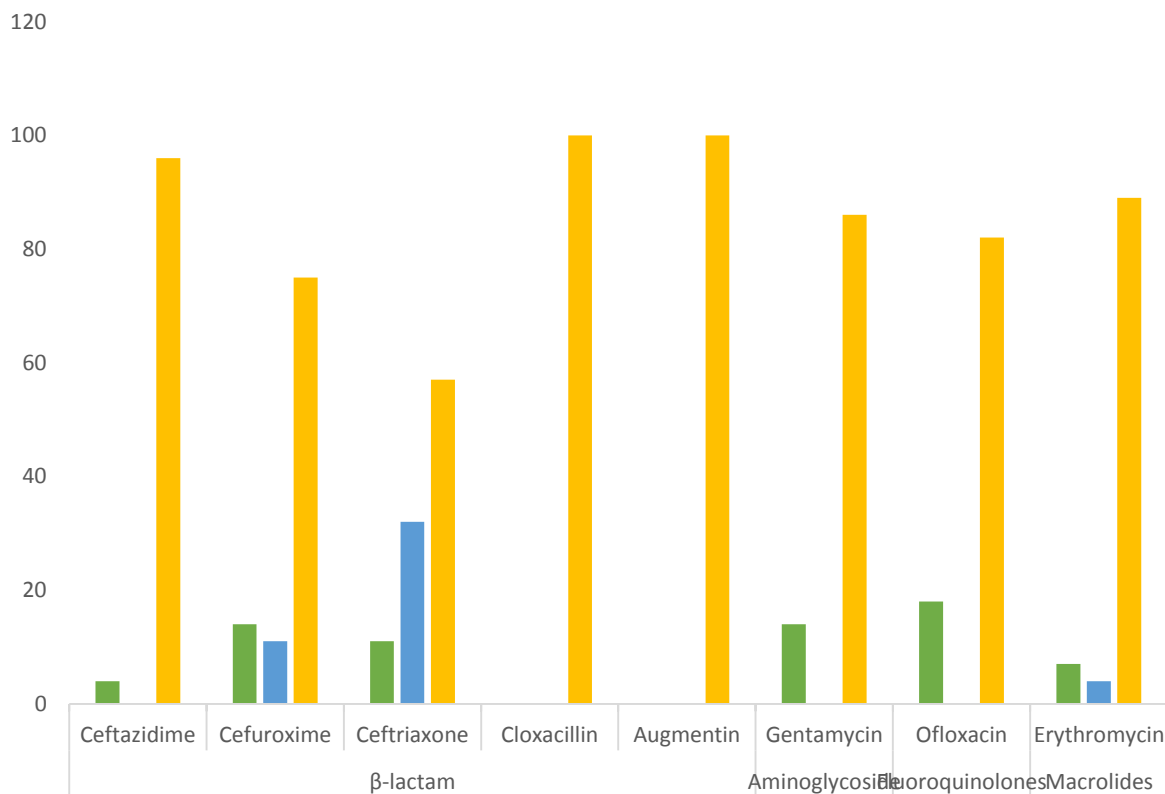
382

383 **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

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

386 18% of the obtained *Staphylococcus aureus* were also resistant to Ceftazidime, Cefuroxime,
 387 Augmentin, Ofloxacin, Cloxacillin, Erythromycin and Gentamicin. The susceptible and
 388 resistant pattern of the antibiotics to *Staphylococcus aureus* according to the classes they
 389 belong to is represented in Figure 1.



390

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

DISCUSSION AND CONCLUSION

392
393 In this study, an incidence of 62.2% *Staphylococcus aureus* was observed on the surfaces of
394 egg shells which is similar to 58.9% reported by Stepien-Pysniak *et al.*, (2009) on surface
395 eggs in Egypt. An incident rate of 100% was also observed by Jehan *et al.*, (2014) on surface
396 eggs in Egypt. This implies that *S. aureus* are more or less frequently found on egg shell
397 surfaces. Sources of *S. aureus* contamination may range from the poultry bird itself, the
398 environment as well as poultry egg handlers and the hygiene practise. The poultry system
399 practised may also serve as a source of horizontal transmission of the organism.
400 Furthermore, *Staphylococcus aureus* on the surface of egg shells are potential microbial
401 source of contamination to the egg content. Wissman (2006) has reported that an embryo can
402 die within 48 hours of exposure to *Staphylococcus aureus*. The presence of *S. aureus* on
403 human skin can also cause cross-contamination and transfer from person to person via
404 contact.

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

416 36% of the isolated *Staphylococcus aureus*, in this study, showed multidrug resistance to the
417 combination of the following antibiotics; Ceftazidime, Augmentin, Ofloxacin, Cloxacillin,
418 Erythromycin, Ceftriaxone, Gentamycin and Cefuroxime. 18% of the isolated *Staphylococcus*
419 *aureus* were also resistant to the combination of Ceftazidime, Cefuroxime, Augmentin,
420 Ofloxacin, Cloxacillin, Erythromycin and Gentamicin. Treatment of infections caused by *S.*
421 *aureus* is often complicated by the high prevalence of multi-drug resistant strains which are a
422 consequence of the indiscriminate and inappropriate use of antimicrobials associated with
423 vertical and horizontal resistance gene transfer (Hiramastu *et al.*, 2013). Microorganisms can
424 survive due to the ability to adapt to antimicrobial agents. They do so via spontaneous
425 mutation or by DNA transfer. This process enables bacteria such as *Staphylococcus aureus* to
426 oppose the action of certain antibiotics rendering the antibiotics ineffective (Bennet, 2008).
427 *Staphylococcus aureus* employs several mechanisms such as efflux mechanisms to remove
428 antibiotics and attaining multi-drug resistance (Li and Nikaido, 2009). Antibiotic resistant
429 bacteria are able to transfer copies of DNA that code for a mechanism of resistance to other
430 bacteria including strains that are distantly related to them. The newly resistant strains are
431 also able to pass on the resistant genes and by so doing generations of antibiotics resistant
432 bacteria are produced (Hussain, 2015).

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

440

441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490

REFERENCES

- Andersson, D.I. (2006). The biological cost of mutational antibiotic resistance: any practical conclusions?. *Current Opinion in Microbiology*. **9**: 461–465.
- Arora, B. and Arora, D. R. (2007). *Practical Microbiology*. Publisher and Distributors, New Delhi. P. 41-42.
- Becker, K., Friedrich, A. W., Lubritz, G., Weilert, M., Peters, G., Von Eiff, C. (2003). Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens". *J. Clin. Microbiol.* **41**:1434–1439.
- Bennet, P. M. (2008). Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistant genes in bacteria. *Br J pharmacol.* **1**: 347-357.
- Brown, A. E. (2005). *Benson's Microbiological Applications*. McGraw-Hill, New York. Pp. 254-258.
- Compassion in World Farming Poultry. Ciwf.org.uk. Retrieved August 26, 2011. Accessed April 14, 2017.
- Curren, J., P., Al-Sahili, F., L. (1980). Neonatal Staphylococcal scalded disease syndrome: massive outbreak due to an unusual phage type. *Paediatrics* **66**: 285-290.
- Davey, P. G. (2000). Concise Oxford Textbook of Medicine. Oxford University Lidingham. p1457
- Dhand, A., Bayer, A. S., Pogliano, J., Yang, S. J., Bolaris, M., Nizet, V., Wang, G., Sakoulas, G. (2011). Use of antistaphylococcal beta-lactams to increase daptomycin activity in eradicating persistent bacteremia due to methicillin-resistant *Staphylococcus aureus*: role of enhanced daptomycin binding. *Clin Infect Dis* **53**:158–163.
- Dingles, M.M., Orwin, P. M, Schlievert, P. M (2000). Exotoxins of *Staphylococcus aureus*. *Clinical Microbiology Reviews*.**13**: 16–34.
- Dyer, B. D. (2003). Pathogens. *A field guide to bacteria*. Cornell University Press. ISBN 978-0-8014-8854-2.
- Fawole, M. A., and Oso, B. A. (2001). Laboratory manual of microbiology. *Spectrum books limited, Ibadan, Nigeria*. Pp 16-24.
- Food and Agricultural Organisation (FAO) of the United Nations (2008). An analysis of the poultry section in Ethiopia. Poultry section country review. Food and Agricultural Organisation. Rome, Italy. Pp:1-48.
- Food and Agricultural Organization (FAO) of the United Nations (2013). Animal production and health. *Poultry and human health* **57**:1-23.
- Hiramatsu, K., Ito, T., Tsubakishita, S., Sasaki, T., Takeuchi, F., and Morimoto, Y., (2013). Genomic basic for methicillin resistance in *Staphylococcus aureus*. *J infect chem.* **45**:117-136.
- Horne, P. L. M., and Van, Achterbosch, T., J. (2008). Animal welfare in poultry production systems: impacts of EU standards on world trade. *World's poultry science journal*. Cambridge University Press (CUP). **64**: 40 - 52.
- Hussain, T. (2015). Pakistan at the verge of potential epidemic by multi-drug resistant pathogenic bacteria. *Advanced Life Science* **2**. Pp: 46 – 47.
- Jarraud S, Peyrat MA, Lim A, et al., (2001). Egc - a highly prevalent operon of enterotoxin gene, forms a putative nursery of super antigens in *Staphylococcus aureus*. *J. Immunol.* **166**: 669–677.
- Jayatilleke, K. and Bandara P. (2010). Antibiotic susceptibility pattern of *Staphylococcus aureus* in tertiary hospital of Sri Lanka. *Sri Lanka Journal of Infectious Diseases* **2012**. **2**:13-17.

- 491 Jehan, I. I., Dalia, M. H. and Husny, A. A. (2014). Prevalence and Inhibition of Microbial
 492 load on Chicken with special reference to egg quality and hatchability. *American*
 493 *Journal of Animal and Veterinary Science*. **9**: 294-302.
- 494 Karama, M., Cencei, G., Rossitto, P. V., Morgante, R. A., and Cullor, J. S. (2003).
 495 Enterotoxins production of *Staphylococcus aureus* isolated from mastitis cow.
 496 *Journal of food production* **66**:9
- 497 Leenstra, F., Napel, J., Ten; Visscher, J., Sambeck, F., and Van, (2016). Layer breeding
 498 programmes in changing production environments: a historic perspective. *World's*
 499 *poultry science journal*. Cambridge University Press (CUP). **72**: 21- 36.
- 500 Levy, S.B (1994). "Balancing the drug-resistance equation". *Trends Microbiol.* **2**:341–342.
- 501 Li, X. Z., Nikaido, H. (2009). Efflux – mediated drug resistance in bacteria: an update.
 502 *Drugs*. **69**: 1555- 1623.
- 503 Meseret, S. (2016). A review of poultry welfare in conventional production system. *Livestock*
 504 *Research for Rural Development*. **28**:12.
- 505 Messens, W., Grijspeerdt, K., De Reu K., De Ketelaere, B., Mertens, K., Bamelis, F., Kemps,
 506 B., De Baerdemaeker, J., Decuyper, E. and Herman, L. (2007). Eggshell penetration
 507 of various types of hen's eggs by *Salmonella enterica* serovar Enteritidis. *Journal of*
 508 *food protection*. **70**:623-628.
- 509 Ogboghodo, I., B., Oviasogie, F., E., Beshiru, A., Omoregie, B., O., Ogofure, P. and Ogofure,
 510 A., G. (2016). The microbial burden load of eggshells from different poultry rearing
 511 systems in Ekosodin Village, Edo State, Nigeria. *Journal of Applied Sciences and*
 512 *Environmental Management*. **20**:227-231.
- 513 Otajevwo, F. D. and Momoh, S. A. (2013). Resistance marker loss of multi-drug
 514 *Staphylococcus aureus* strains. *Journal of Applied Medical Sciences* **2**:43-62.
- 515 Pearson, C. (2007). Antibiotic Resistance Fast-Growing Problem Worldwide. *Voice of*
 516 *America*. Archived from the original on 2 December 2008. Retrieved 29
 517 December 2008. Accessed 28 May 2017.
- 518 Public Health England (PHE), (2014). *Indole test UK standards for microbiology*
 519 *investigation*. **19**: 1-14.
- 520 Rasmussen, R., Y., Fowler, V., G., Skov, R., Brunn, N., E. (2011). Future challenges and
 521 treatment of *Staphylococcus aureus* bacteremia with emphasis on MRSA. *Future*
 522 *microbiology* **6**: 43-56.
- 523 Ryan, K. J., Ray, C. G. (2004). *Sherris Medical Microbiology* (4th ed.). McGraw
 524 Hill. [ISBN 0-8385-8529-9](#).
- 525 Smith, A. J. (2001). *Poultry: The Tropical Agriculturist. Revised edition, Published by*
 526 *Macmillan Education Ltd, London and Oxford, UK*. Pp. 218.
- 527 Smith, A., Rose, S., P., Wells, R., G. and Pirgozliev, V. (2000). The effect of changing the
 528 excreta moisture of caged laying hens on the excreta and the microbial contamination
 529 of their egg shells. *British Poultry Science*. **41**:168-173.
- 530 Sparks, N. H. (2006). The hens egg – is its role in human nutrition changing?. *World's*
 531 *poultry science journal* **62**: 308-315.
- 532 Stepien-Pysniak, D., Marek, A. R. and RzedzickI, J. (2009). Occurrence of bacteria of the
 533 genus *Staphylococcus* in table eggs from different sources. *Pol. J. Veterinary Science*.
 534 **12**:481- 484.
- 535 Tiwari, R. P., Hoondal, G. S., and Tewari, R. (2009). Laboratory techniques in microbiology
 536 and biotechnology. *Abhishek publications, India*. P. 75-81.
- 537 Todar, K. (2008). *Online textbook of bacteriology*. University of Wisconsin, Madison. **Pp** 1-6
- 538 Tong, S., Y., Davis, J., S., Eichenberger, E., Holland, T., L., Fowler, V., G. (2015).
 539 *Staphylococcus aureus* infections, epidemiology, pathophysiology, clinical
 540 manifestations and management. *Clinical microbiological reviews*. **28**: 603-661.

- 541 Topp, E., Chapman, R., Devers-Lamrani, M., Hartmann, A., Marti, R., Martin-Laurent, F.,
542 Sabourin, L., Scott, A. and Sumarah, M. (2013). Accelerated Biodegradation of
543 Veterinary Antibiotics in Agricultural Soil following Long-Term subst: lc :Exposure
544 and Isolation of a Sulfamethazine-degrading spices. *J. Environ. Qual.* **42**: 173–178.
- 545 Varrone, J. J., De Mesy Bentley K. L., Bello-Irizarry, S. N., Nishitani, K., Mack, S., Hunter
546 J. G., Kates, S. L., Daiss, J. L., Schwarz, E. M. (2014). Passive immunization with
547 anti-glucosaminidase monoclonal antibodies protects mice from implant-associated
548 osteomyelitis by mediating opsonophagocytosis of *Staphylococcus aureus*
549 megaclusters. . *Journal of Orthopedic Research.* **32** (10): 1389–1396.
- 550 Von Nussbaum, F., Brands, M., Hinzen, B., Weigand, S. and Habich, D. (2006).
551 Antimicrobial Natural Products in Medical Chemistry – Exodus or Revival?.
552 *Angewandte Chemie International Edition.* **45**:5072 – 5129.
- 553 Windhorst, H. W. (2008). A projection of the regional development of egg production until
554 2015. *World's poultry science Journal* **64**: 356-376.
- 555 Wissman, M. A. (2006). Diseases transmitted to eggs. www.exoticpetvet.net
- 556 Witte, W. (2004). "International dissemination of antibiotic resistant strains of bacterial
557 pathogens". *Infect. Genetic Evolution.* **4**: 187–191.