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# Isolation and characterization of bacteria associated wit h yolk sac infection (Omphalitis) in chicken from three hatcheries in Bishoftu, Ethiopia

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**A study was conducted from November 2014 to June 2015 at three hatcheries designated as A, D and E in Bishoftu Town, Ethiopia, to isolate and identify bacteria associated with yolk sac infection and to determine antimicrobial susceptibility profile of the predominant isolates. A total of 385 Lohmann and Koekoek breed, 1 to 7 days old chicks suffering from yolk sac infection were examined from three different hatcheries of which 96.1% (N=370) of them were showing unabsorbed yolk sac. All the chicks were necropsied and yolk sac samples were collected for isolation and identification of bacteria followed by testing of the isolates for their susceptibility to 11 antimicrobial agents using disc diffusion method. A total of 323 bacterial isolates were identified, of these *Escherichia coli* were the most common bacteria (N=116; 35.91%) isolated followed by Salmonella species (N=111; 34.36%) and *Staphylococcus aureus* (N=96; 29.72%). Significant difference (P<0.001) was noted among the hatcheries on the frequency of isolation of the predominant bacteria species from yolk sac samples with the highest rate of isolation being in hatchery A. All the tested predominant bacterial isolates showed higher susceptibility to Gentamicin, Chloramphenicol, Amikacin (except *S. aureus*) and Kanamycin but were resistant to Ciprofloxacin, (except *S. aureus*), Penicillin G, Tetracycline, Sulfamethoxazole and Amoxicillin (except *S. aureus*). The existence of multi-drug resistant bacteria isolates associated with yolk sac infection suggests that more emphasis should be given towards preventing omphalitis in chicks through improvements of sanitary measures at hatcheries than to use antimicrobials to control infections.**

**Key words:** Bacteria, chicken, yolk sac infection, antimicrobial sensitivity, Ethiopia.

## INTRODUCTION

The rapid expansion of poultry industry has presented many poultry diseases. Yolk Sac Infection (YSI) also known as mushy chick disease or omphalitis is one of the economically important diseases of poultry. The affected chicks manifest depression, drooping of the head and huddling near to the heat source (Kahn et al., 2008).

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Several bacteria such as *Escherichia coli, Salmonella* species*, Proteus* species*, Enterobacter* species*, Pseudomonas* species*, Klebsiella* species*, Staphylococcus* species*, Streptococcus* species*, Clostridium* species*, Bacillus cereus* and *Enterococcus* have been isolated from the yolk sac of the infected birds in several studies (Corts et al., 2004; Iqbal et al., 2006). An outbreak of YSI caused by *Klebsiella pneumonia* in 1 to 3 day old canary chicks (*Serinus canaria*) have been reported recently (Razmyar and Zamani, 2016).

YSI is responsible for increased mortality in week-old chicks, poor weight gain, retarded growth and poor carcass quality in surviving birds (Corts et al., 2004). It may also result in decreased hatchability and increased culling rate due to retarded growth. It is accounting for large economic losses to the poultry industry with mortality rates reaching 5 to 10% (Ulmer, 2011). In Ethiopia, yolk sac infections (Omphalitis) have got little attention. Reports from Kombolcha hatchery in Ethiopia showed the significance of the problem with a prevalence rate of 33.1% (Abadi et al., 2013). Thus, the objectives of this study were to isolate and identify the bacteria from clinical cases of omphalitis (YSI) in newly hatched chicks from three selected hatcheries in Bishoftu Town and to determine their susceptibility against most commonly used antimicrobials.

## MATERIALS AND METHODS

### Study area

The study was conducted on three selected hatcheries located in Bishoftu Town between November 2014 and June 2015. Bishoftu is located at 47 km Southeast of Addis Ababa at an altitude of about 1900 masl (38° 58″ E 08° 44″ N) harboring a number of commercial and small scale poultry farms. It has an annual rainfall of 1115.6 mm (NMSA, 2003). The three hatcheries were considered in this study due to the ease of access for sample collection and were designated as hatchery A, E and D for the purpose of this study.

### Study animals and sampling

The chicks included in the current study were obtained from three successive batches of parent flocks of Lohmann (from hatcheries A and E) and Koekoek (Hatchery D) breeds. The chicks were physically examined for any signs of yolk sac infection with particular attention to the umbilical area and those with such signs were considered for further investigation. A total of 385 chicks (1 to 7 days old) showing signs of YSI were necropsied and examined for any gross lesions extending to the visceral organs with special reference to the yolk sac according to the procedures described previously (Chauhan and Roy, 2007). For bacteriological examination, yolk sac samples were collected aseptically using sterile plain swabs which were then labeled, packed and transported in portable coolant (ice pack) to Debre-zeit Agricultural Research Center Animal health laboratory. The collected samples were stored in refrigerator at 4°C until bacteriological analysis.

### Bacterial isolation

Swab samples were aseptically streaked on to blood and MacConkey (Oxoid, UK) agar plates and incubated at 37°C for 16 to 40 h (Quinn et al., 2002) after which the plates were examined for any growth of bacteria. Based on macroscopic appearance, each of the different types of colonies observed were picked and sub-cultured to get pure cultures of each isolate. The isolates obtained were then streaked onto several selective agar plates including eosin methylene blue agar (EMB), brilliant green agar (BGA), mannitol salt agar (MSA), *Salmonella*-Shigella (SS) agar and Harlequin *Salmonella* ABC medium and incubated at 37°C for 16 to 40 h for further characterization.

### Bacterial identification

Presumptive identification of bacterial isolates was done based on colony morphology (size, margin, elevation and color), Gram stain reaction and cellular morphology (Merchant and Packer, 1967) and biochemical characteristics using catalase, coagulase, M-R, V-P, Indole, triple sugar iron (TSI) agar and sugar fermentation tests as described previously (Swayne et al., 1998; Quinn et al., 2002; Cheesbrough, 2006).

### Antimicrobial susceptibility test

Antimicrobials used for the current susceptibility test were selected based on their common application in the treatment of infections in poultry in Ethiopia which included Gentamicin, Amoxicillin, Penicillin, Kanamycin, Norfloxacin, Streptomycin, Chloramphenicol, Ciprofloxacin, Tetracycline, Sulfamethoxazole and Amikacin. Antimicrobial susceptibility was done by employing the disc diffusion or Kirby-Bauer method (Bauer et al., 1966). Briefly, an inoculum was prepared from an overnight culture of each bacterial isolate by suspending in sterile saline solution adjusted to 0.5 McFarland turbidity standard. Each isolate was inoculated onto previously prepared Mueller Hinton agar (Oxoid, UK) by streaking the whole surface of the plate with sterile cotton swab in a way to get evenly distributed confluent colonies. The inoculated MH plates were then made to dry and discs of selected antimicrobials were placed approximately 2.5 cm apart and gently pressed using sterile forceps. Plates were then incubated for 24 to 28 h at 37°C after which the diameter of zone of inhibition is measured and recorded. Determination of zone-size break points for defining the susceptible, intermediate and resistant categories for an antimicrobial agent was performed according to the established standards (NCCLS, 2007).

### Data analysis

The laboratory result was coded and managed into Microsoft Excel and analyzed using statistical package for social sciences (SPSS) version 16. Descriptive analysis such as sum and percentages were used in summarizing the results. Chi-square test of independence was employed at 95% confidence level to determine whether significant differences exist in the rate of bacterial isolation among the three hatcheries.

## RESULTS

Upon physical examination, all the birds included in the current study had the typical signs of omphalitis with a characteristic markedly thickened and dark blue navel, distended and soft abdomen.

At necropsy, the major gross lesions observed in chicks

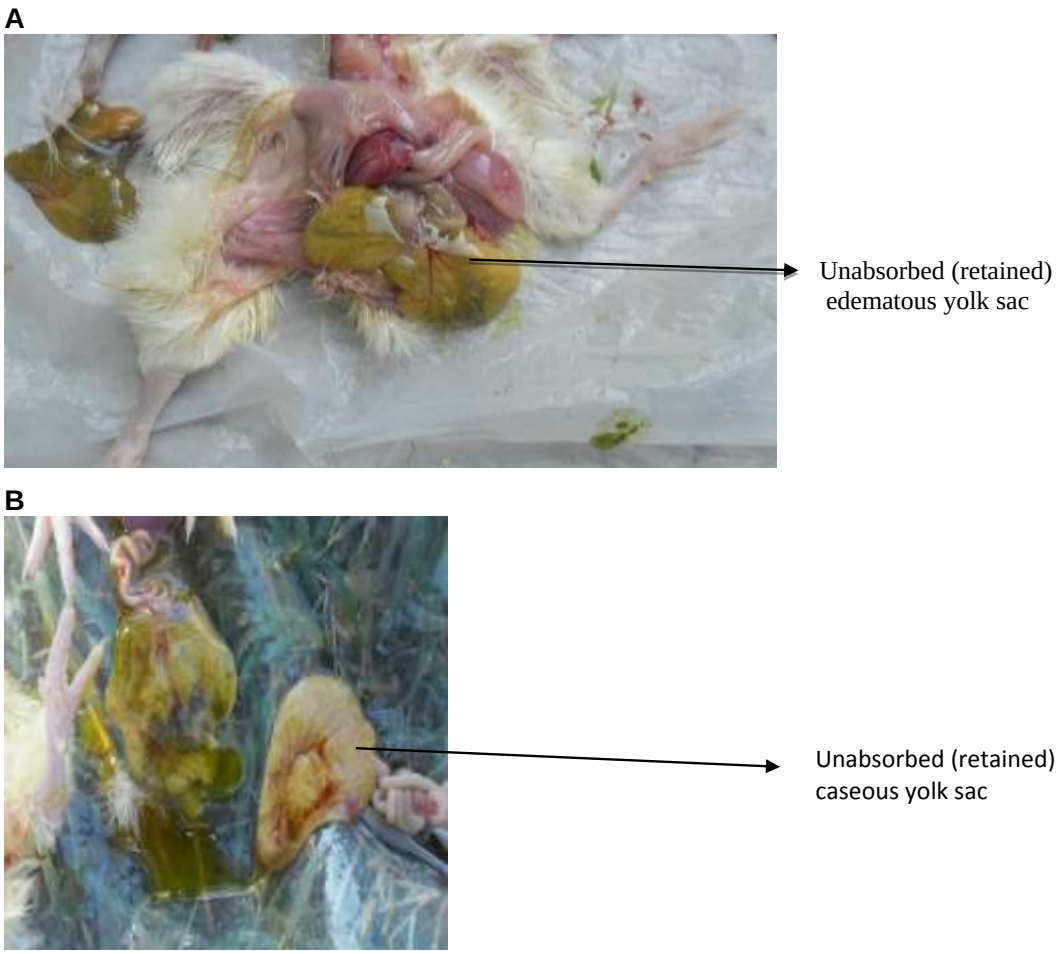


**Figure 1.** (A and B) Unabsorbed yolk sac in day-old chick.

with yolk sac infection were unabsorbed yolk sac; in many cases (370 out of 385) congestion and discoloration of the yolk (greenish yellow; dark brown to bright yellow), retained caseous yolk sac and edematous yolk (especially in 3 to 7 days old chicks) (Figure 1A and B). Peritonitis, pericarditis, petechial and ecchymotic hemorrhages on the serosal surface of visceral organs (particularly of the intestine) were also observed.

Bacterial isolation and identification from yolk sack specimens showed three predominant bacteria species which included *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* based on cultural, morphological and biochemical features which were consistent with the characteristics of the respective bacterial species (Table 1). The frequency of isolation of the predominant bacterial species from cases of omphalitis from the three hatcheries is as shown in Table 2. In all cases of yolk sac infections, more than one type of bacteria species was isolated revealing that mixed infection is a common scenario. Out of the total 323 different bacteria isolates belonging to the different genera obtained from cases of yolk sac infections from the three hatcheries, *E. coli* (N=116; 35.491%) was the most predominant isolate followed by *Salmonella* spp. (N=111; 34.36%) and *S. aureus* (N=96, 29.72). Significant difference (P<0.001) was observed among the hatcheries on the frequency of isolation of the predominant bacteria species from yolk sac samples with the highest rate of isolation being in hatchery A (Table 2).

Further, *in vitro* antimicrobial susceptibility test of the three predominant bacteria species using 11 different antimicrobials showed higher susceptibility to Gentamicin, Chloramphenicol, Amikacin (except *S. aureus*) and kanamycin. With the exception of *S. aureus* which was susceptible to Ciprofloxacin and Amoxicillin, all the isolates of the three bacterial species were found resistant to Ciprofloxacin, Penicillin G, Tetracycline, Sulfamethoxazole and Amoxicillin (Table 3).

## DISCUSSION

Yolk sac infection is one of the health problems of poultry responsible for considerable losses. It results in

**Table 1.** Cultural and biochemical characteristics of representative predominant bacteria isolates from yolk sac infection in young (1-7 day old) chicken.

| Characteristic | Representative bacteria isolates | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| **Growth in** | | | |
| EMB | + | - | - |
| BA | + | + | + |
| BGA | + | + | - |
| MSA | - | - | + |
| XLD | + | + | - |
| SSA | + | + | - |
| Harlequin | - | + | - |
| MacConkey | + | + | - |
| **Biochemical tests** | | | |
| MR | + | + | + |
| VP | - | - | + |
| Indole | + | - | - |
| Catalase | + | + | + |
| TSI, Yellow slant, Butt with gas | + | - | - |
| TSI, Butt yellow, slant pink | - | + | - |
| $H_2S$ | - | + | - |
| Carbohydrate fermentation tests | DX, AG | DX, AG | DX, A |
| | SU, AG | MN, AG | SU, A |
| | L, AG | ML, AG | L, A |
| | MN, AG | - | MN, A |
| | - | - | ML, A |
| Interpretation | *E. coli* | *Salmonella* spp. | *S. aureus* |

EMBA: Eosin methylene blue agar; BA: blood agar; BGA: brilliant green agar; MSA: mannitol salt agar; XLD: xylose-lysine deoxycholate agar; SSA: *Salmonella* Shigella agar; DX: dextrose; ML: maltose; L: lactose; SU: sucrose; MN: mannitol; A: acid; AG: acid and gas; +: Positive, –: Negative; MR: Methyl red; VP: Voges-Proskauer; TSI: triple sugar iron agar; $H_2S$: hydrogen sulphide production.

**Table 2.** Frequency of isolation of predominant bacterial species from cases of yolk sac infections in three hatcheries.

| Isolate | Hatchery | No. examined | N (%) | $\chi^2$ | *p*-value |
|---|---|---|---|---|---|
| *Escherichia coli* | A | 235 | 57 (24.3) | 45.456 | 0.000 |
| | D | 37 | 29 (78.4) | | |
| | E | 113 | 30 (26.5) | | |
| *Salmonella* spp. | A | 235 | 52 (22.1) | 36.119 | 0.000 |
| | D | 37 | 26 (70.3) | | |
| | E | 113 | 33 (29.2) | | |
| *Staphylococcus aureus* | A | 235 | 40 (17.0) | 48.648 | 0.000 |
| | D | 37 | 26 (70.3) | | |
| | E | 113 | 30 (26.5) | | |

decreased hatchability, increased mortality and culling rate in affected flocks due to retarded growth following alteration in structure of immunoglobulin proteins accompanied by microbial infection subsequently resulting in immunosuppression (Sander et al., 1998). It occurs mainly due to bacterial contamination of the egg

**Table 3.** Antimicrobial susceptibility pattern of the most frequently isolated bacteria involved in yolk sac infection in chicken.

| Antimicrobial agents | Disc content (µg) | *Escherichia coli* isolates (n=116) | | | *Salmonella* spp. isolates (n=111) | | | *Staphylococcus aureus* isolates (n=96) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | R (%) | I (%) | S (%) | R (%) | I (%) | S (%) | R (%) | I (%) | S (%) |
| Gentamicin | 10 | 0 (0) | 0 (0) | 116 (100) | 0 (0) | 20 (18.0) | 91 (81.9) | 0 (0) | 0 (0) | 96 (100) |
| Ciprofloxacin | 5 | 116 (100) | 0 (0) | 0 (0) | 111 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 96 (100) |
| Penicillin G | 10u | 116 (100) | 0 (0) | 0 (0) | 111 (100) | 0 (0) | 0 (0) | 70 72.9) | 26 (27.0) | 0 (0) |
| Tetracycline | 30 | 116 (100) | 0 (0) | 0 (0) | 111 (100) | 0 (0) | 0 (0) | 96 (100) | 0 (0) | 0 (0) |
| Sulfamethoxazole | 25 | 116 (100) | 0 (0) | 0 (00) | 111 (100) | 0 (0) | 0 (0) | 96 (100) | 0 (0) | 0 (0) |
| Amikacin | 30 | 0 (0) | 0 (0) | 116 (100) | 0 (0) | 0 (0) | 111 (100) | 24 (27.0) | 70 (72.9) | 0 (0) |
| Chloramphenicol | 30 | 0 (0) | 0 (0) | 116 (100) | 0 (0) | 0 (0) | 111 (100) | 0 (0) | 0 (0) | 96 (100) |
| Amoxicillin | 10 | 116 (100) | 0 (0) | 0 (0) | 111 (100) | 0 (0) | 0 (0) | 0 (0) | 20 (20.8) | 76 (79.1) |
| Kanamycine | 30 | 0 (0) | 0 (0) | 116 (100) | 0 (0) | 0 (0) | 111 (100) | 10 (10.6) | 0 (0) | 84 (89.4) |
| Norfloxacin | 10 | 116 (100) | 0 (0) | 0 (0) | 111 (100) | 0 (0) | 0 (0) | 26 (27.0) | 0 (0) | 70 (72.9) |
| Streptomycin | 10 | 0 (0) | 116 (100) | 0 (0) | 11 (9.9) | 100 (90.0) | 0 (0) | 0 (0) | 0 (0) | 96 (100) |

shell after the egg is laid, while the cuticle is still moistened. Factors promoting contamination include lack of hygiene in the nests, presence of eggs on the floor, incubation of dirty eggs or eggs with egg shell defects and collection of dirty and clean eggs at the same time (Rahman et al., 2007; Ahmed, 2009; Ulmer, 2011).

The current study carried out on the isolation of bacterial agents associated with yolk sac infection (omphalitis) in three hatcheries in Bishoftu is the first report in the study area which showed the importance of yolk sac infection as the cause of high mortality in chicks during their first week of life.

The findings of the three predominant bacterial species (*E. coli, Salmonella* and *S. aureus*) isolated from yolk sac infection in this study are corroborated by several earlier studies which documented the frequent association of these bacteria species with yolk sac infections (Amer et al., 2017; Abdel-Tawab et al., 2016; Hazariwala et al., 2002; Rosario et al., 2004; Iqbal et al., 2006; Buhr et al., 2006; Suha et al., 2008).

*E. coli* has been previously reported as one of the most frequently isolated bacteria involved in the development of yolk sac infection (omphalitis) (Amer et al., 2017; Abdel-Tawab et al., 2016; Buhr et al., 2006; Suha et al., 2008). *S. aureus* has also been reported as an important cause of diseases in poultry (Hazariwala et al., 2002) as well as a common cause of yolk sac infection in broilers (Amer et al., 2017; McCullagh et al., 1998). Involvement of *Staphylococcus*, *Proteus*, *Streptococcus* and *bacillus* species has also been reported previously (Amer et al, 2017; Rosario et al., 2004; Buhr et al., 2006; Suha et al., 2008).

Several other species of bacteria have also been reported from cases of yolk sac infection in chicks including *Salmonella*, *Staphylococcus, Protease*, *Bacillus, Streptococcus, Pseudomonas, Klebsiella, Clostridium, Aerobacter, Citrobacter, Achromobacter,* and *Enterococci* spp. (Amer et al., 2017; Anjum, 1997; Deeming, 1995; Sainsbury, 1992). Involvement of *Aspergillus fumigatus* in yolk sac infection was also reported by Schonhofen and Garcia (1981).

The relatively few bacteria species isolated in the current study may be due to the use of antimicrobial agents by some of the poultry farms to control early chick mortality.

Consistent to the current study, the gross lesions observed in chicks suffering from yolk sac infection which were manifested as edematous and unabsorbed/retained yolk sac was also reported by different workers (Suha et al., 2008; Ahmed et al., 2009; Kawalilak et al., 2010).

Several previous studies indicated that prolonged storage of eggs, improper temperatures and humidity during incubation as predisposing factors contributing to increased incidence of YSI. Too high or low incubation temperature during the final days of incubation will produce poorly closed navels. When eggs are stored for prolonged periods prior to incubation, more chicks with black scab navels are observed, indicating unhealed navels at the moment of hatching. Too high humidity during incubation results in insufficient weight loss. As a result, the residual yolk sac becomes enlarged, which prevents the navel from

closing properly. Conversely, when humidity is too low, the yolk sac dehydrates and becomes hard, which can damage sensitive tissue around the navel (Sainsbury, 1992; Sarma et al., 1985; Saif et al., 2003). Improper management associated with the conditions of egg storage and incubation may have played a role in predisposing young chicks for yolk sac infection observed in the current study requiring further investigation into the specific management practices in the hatcheries to have conclusive remark.

The higher susceptibility of bacterial isolates obtained from YSI to antimicrobials such as Gentamicin, Chloramphenicol, Amikacin and Kanamycin in *in-vitro* drug sensitivity test were in agreement with the previous reports (Salehi and Bonab, 2006; Sharada et al., 2010) indicating their potential application for effective treatment of diseases caused by these bacteria species. The resistance of *E. coli, Salmonella* and *S. aureus* to multiple antimicrobials observed in this study supports the reports of previous works (Khan et al., 2002; Nasrin et al., 2012; Lee et al., 2005; Abadi et al., 2013). This may probably suggest the wide use of these antimicrobials for treatment of bacterial infections in both animals and humans as these bacteria are environmental contaminants from clinical cases. This necessitates establishing alternative efficient strategies for the prevention and control of bacterial omphalitis through careful improvements of sanitary measures in hatcheries.

In conclusion, the results of the present study in Bishoftu hatcheries from cases of omphalitis in chicks show that *E. coli, Salmonella* spp. and *S. aureus*, are the predominant bacteria isolated from yolk sack samples indicating that these bacteria species are the major cause of yolk sac infection.

Antimicrobials such as Gentamicin, Chloramphenicol, Kanamycin and Amikacin may be potentially effective for treatment of yolk sac infection in chicks. However, the isolation of multi-drug resistant strains of *E. coli, S. aureus* and *Salmonella* spp. from cases of chicks suffering from omphalitis is alarming as this resistance may spread to microbes infecting man and animals.

The findings from the current study signify the importance of the problem requiring more detailed study to determine the extent of the problem in different poultry farms, the role and pathogenicity of each bacterial species involved in yolk sac infection as well as to explore possible predisposing factors.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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