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## Resistance patterns associated with bacterial pathogens causing omphalitis in baby chicks

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A study was conducted on 200 samples of one day old chicks obtained from domestic & imported chicken hatchlings to isolate and identify bacteria associated with yolk sac infection (Omphalitis) and to determine antimicrobial sensitivity pattern of the predominant bacterial pathogens. A total of 160 bacterial isolates were isolated and identified using biochemical tests and molecular confirmation. The bacterial strains tested for their susceptibility to 10 antimicrobial agents. The highest recovery rate was for *Escherichia coli* 120 (60%) followed by *Pseudomonas aeruginosa* 25 (12.5%). *Staphylococcus aureus* and *Salmonella* were 10 (5%) & 5 (2.5%), respectively. The antimicrobial sensitivity patterns were detected for all bacterial genera; the highest resistance patterns were exhibited by *P. aeruginosa* followed by *Salmonella* spp. then *E. coli* and finally *S. aureus*. The existence of multi-drug resistance bacteria isolates associated with yolk sac infection suggests that more emphasis be given towards preventing omphalitis in chicks through improvements of sanitary measures than to consider control options through the use of antimicrobials

**Keywords:** Omphalitis, Yolk sac, *E. coli*, Bacterial Causes, Baby chicks, Egypt

### INTRODUCTION

Omphalitis is an infectious and noncontagious condition of yolk sac which accompanied by unhealed navels (Rahman et al. 2007). Omphalitis is an economically important disease as it causes first week mortality and causes poor weight gain. In addition, birds those survive to yolk sac infection show poor carcass quality (Corts et al. 2004)

Omphalitis occurs mainly due to unhygienic condition at hatcheries and / or bacterial contamination of the eggshell at the broiler breeder farm from poultry houses litter. The litter can be act as the major source of bacterial & fungal contaminants (Ahmed et al. 2012). Bacterial infection of navel area is one of the most

common causes of mortality in baby chicks during the first week after hatching (Pattison et al. 2008) Many bacterial species could be isolated from yolk sac infection of birds such as *Proteus* spp., *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* spp., *Streptococcus* spp., *Clostridium* spp., *Bacillus cereus* and *Enterococcus* (Cortes et al. 2004).

Bacterial omphalitis or yolk sac infection occurs frequently in commercial poultry. The most prevalent bacteria causing yolk sac infection is *E. coli* representing 70% of omphalitis causes (Saif et al. 2008). *S. aureus* is the next most important bacterium could be associated with yolk sac infection (Deeming, 1995). *S. aureus* is a very ubiquitous microorganism associated with

omphalitis, yolk sac and liver infections in first week dead chicks and in-shell dead embryos (White et al; 2003) the birds of group infected with staphylococcus seemed weak huddled together and had a watery diarrhea.

All over the observation of bacterial infection outbreaks prompted flock surveillance programs for detection and rapid investigation by molecular characterization of circulating bacterial strains (Khalifa et al. 2014). The aim of present study the accurate detection of bacteria pathogens associated with yolk sac infection to undertake appropriate control measure as well as use of appropriate antibiotics to reduce mortality in baby chicks

## MATERIALS AND METHODS

### Bacterial Isolation

Examination of 200 chick's samples, which collected from imported and local chicks, examined yolk sac samples. The samples were submitted to reference laboratory for veterinary quality control on poultry production, Giza, Egypt. The phenotypic characterization and Identification of bacterial genera was done according to standard methods (Quinn et al. 1994, Lee and Arp 1998).

### Direct PCR and molecular confirmatory identification:

The positive samples for bacterial isolation were exposed for direct PCR for confirmatory identification of each suspected bacterial species. The yolk sac samples were processed according Samir et al. 2015. The direct PCR was performed using (Phire Animal Tissue direct PCR Kit) according Hossam et al. 2016. The primer pairs which were used for identification of common bacteria associated with omphalitis were; *eae* for *E. coli* (Osman et al. 2012), *invA* for Salmonella spp. (Rhan et al. 1992), *nuc* for *S. aureus* (Brakstad et al. 1996) and *groE* for *Pseudomonas* (Clarke et al. 2003).

### Serotyping

Serological identification of *E. coli* was done using polyvalent and monovalent slid agglutination sera grouping according to (Lee et al. 2009) .

### Antibiotic sensitivity test:

Antibiotic susceptibility tests were performed for all bacterial isolates by using the standard disc diffusion method. The standard procedures of the CLSI, 2015 method was strictly followed (CLSI,

2015), accordingly the antimicrobial susceptibility of bacterial strains were tested against 10 antimicrobial drugs: tetracycline, streptomycin, ciprofloxacin, norfloxacin, gentamycin, nalidixic acid, ampicillin , erythromycin, sulphamethazone and amoxicillin

## RESULTS

In the present study, the major bacterial species isolated from yolk sac were *E. coli*, *P. aeruginosa*, *S. aureus* and Salmonella spp .based on typical phenotypical features which were consistent with the characteristics of the respective bacterial species presented. The direct PCR on yolk sac for molecular confirmatory identification were applied. All *E. coli* positive samples produced 384 bp products for *intimin* gene (Fig. 1).

Accordingly the total 160 different bacterial strains isolated were confirmed as; *E. coli* 120 (60%) was the most predominant isolate followed by followed by *P. aeruginosa* 25 (12.5%) and *S. aureus* 10 (5%) whereas, Salmonella spp. 5 (2.5%) were the least frequently isolated bacterial species

### Serological identification:

The most commonly detected *E.coli* serogroups were O146, O125, O111, O126, O55, O119, and O168 as shown in table (1)

**Table (1): The distribution of *E.coli* isolates serotypes:**

<i>E. coli</i> Serotype	No of isolate	%
O125	65	54.1
O146	10	8.3
O111	10	8.3
O126	5	4.1
O159	5	4.1
O114	5	4.1
O55	5	4.1
O15	5	4.1
O168	5	4.1
O119	5	4.1
Total	120	100

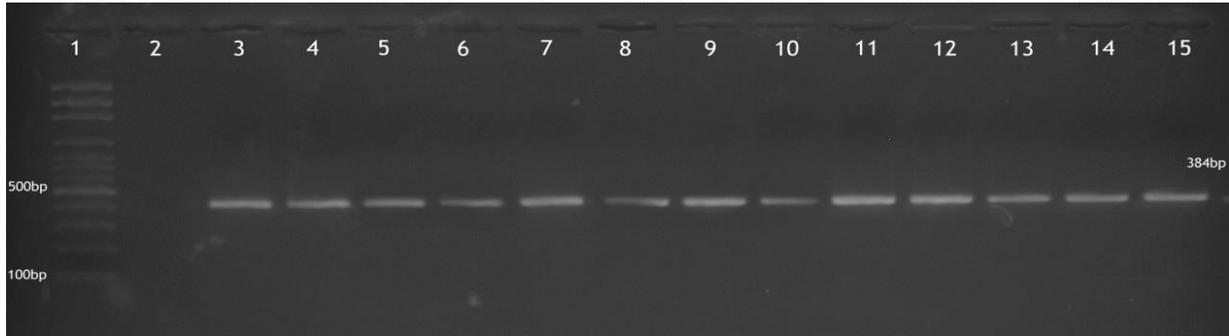
### Antibiotic sensitivity test

In the current study, the intermediate results in antibiotic sensitivity testing were considered as resistance. Antimicrobial sensitivity study of the isolates using 10 different antimicrobials showed that, *E. coli* isolates were susceptible with variable percent to ciprofloxacin, nalidixic acid and

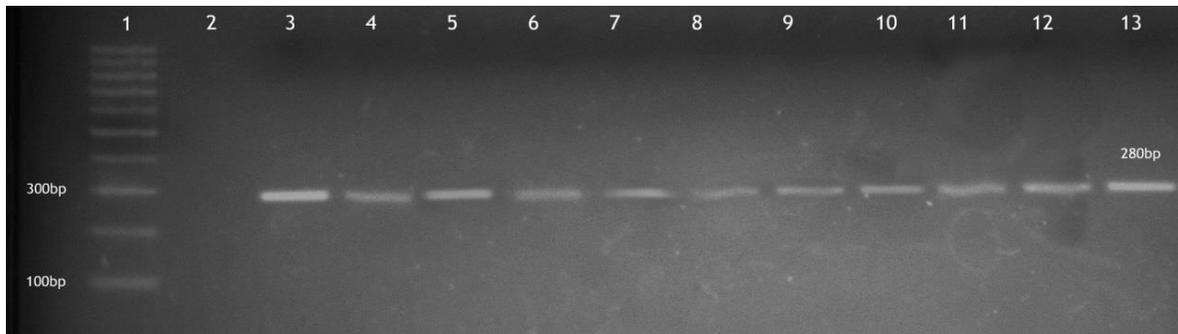
norfloxacin. *S. aureus* isolates showed more similar results to *E. coli* as it were highly susceptible to amoxicillin, ciprofloxacin, gentamicin, tetracycline, erythromycin and norfloxacin. The highest resistance patterns were observed for *P. aeruginosa* & *Salmonella* spp isolates. Most of examined isolates showed

resistance percent 100% to most of tested antimicrobial drugs. The *S. aureus nuc* genes were amplified with 280bp products (Fig. 2).

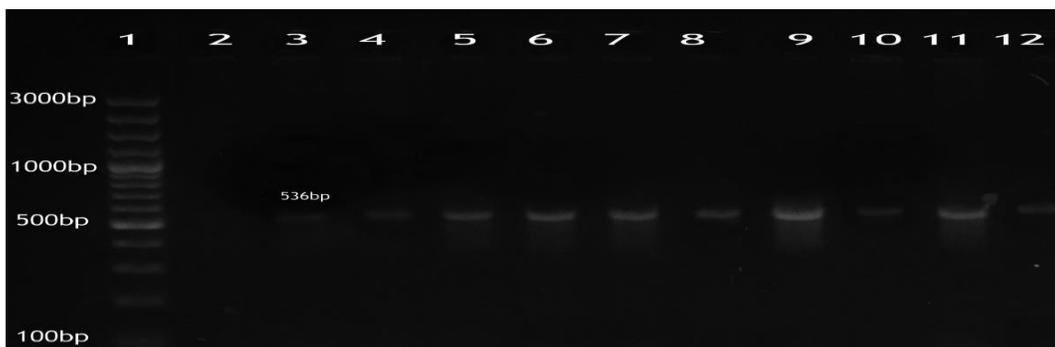
All *P. aeruginosa* produced 536 for *groE* (Fig 3), while *Salmonella* spp isolates were positive for *invA* gene 270 bp (Fig 4).



**Figure 1** Electrophoretic profile of positive *E. coli* yolk sac samples producing 384 bp amplicons for *eae* gene M.: DNA ladder 100 bp (GeneRuler, Thermo)



**Figure 2** Electrophoretic profile of positive *S. aureus* yolk sac samples producing 280 bp amplicons for *nuc* gene M.: DNA ladder 100 bp (Jena Bioscience, Germany)



**Figure 3** Electrophoretic profile of positive *P. aeruginosa* yolk sac samples producing 536 bp amplicons for *groE* gene M.: DNA ladder 100 bp wide range(Jena Bioscience, Germany)

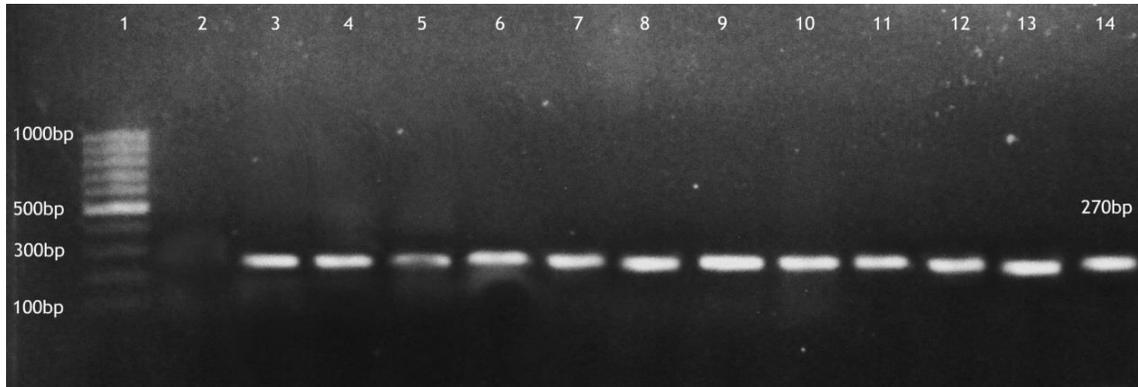


Figure 4. Electrophoretic profile of positive *Salmonella* species yolk sac samples producing 270 bp amplicons for *invA* gene M.: DNA ladder 100 bp (Jena Bioscience, Germany)

Table . 2 Antimicrobial Susceptibility pattern of the most frequently isolated bacteria involved in yolk sac infection in chicken

	<i>E. coli</i>		<i>P. aeruginosa</i>		<i>S. aureus</i>		Salmonella spp	
	S	No (%) R	S	No (%) R	S	No (%) R	S	No (%) R
Amoxicillin	-	120 (100)	-	25 (100)	10	-	-	5 (100)
Ampicillin	-	120 (100)	-	25 (100)	-	10 (100)	-	5 (100)
Ciprofloxacin	95	25 (20)	25	-	10	-	-	5 (100)
Nalidixic acid	95	25 (20)	-	25 (100)	-	10 (100)	-	5 (100)
Gentamicin	110	10 (8)	-	25 (100)	10	-	-	5 (100)
Streptomycin	70	50 (41.6)	-	25 (100)	5	5 (50)	5	-
Tetracycline	30	90 (75)	-	25 (100)	10	-	5	-
Erythromycin	-	120 (100)	-	25 (100)	10	-	-	5 (100)
Norfloxacin	105	15 (12.5)	20	5	10	-	5	-
Trimethoprim-sulfamethoxazole	60	60 (50)	-	25 (100)		10 (100)		5 (100)

## DISCUSSION

Yolk sac infection or omphalitis is one of the health problems of poultry industry including; decreased hatchability, increased mortality and increased culling rate in affected flocks (Yassin et al. 2009). It occurs mainly due to unhygienic condition at hatcheries and / or bacterial contamination of the eggshell at the broiler breeder farm from poultry houses litter. (Ahmed, 2012).

Many studies confirmed that *E. coli* is as one of the most frequently isolated organisms involved in omphalitis (Pattison et al. 2008). Also, *S. aureus* is next most important bacterium associated with yolk sac infection in poultry (Hazariwala et al. 2002). In the present study, the highest recovery rate was for *E. coli* 120 (60%) from baby chick samples. More near percent of *E. coli* recovery from imported baby chicks were reported (Zhao et al. 2001).

The serological typing of isolated *E. coli* in the

present study revealed variable serotypes of *E. coli*. The most commonly detected *E. coli* serogroups were O125 (54.1%), O146 & O111 (8.3%), O126 & O159 & O114 & O55&O168 & O119 and O15 (4.1%), table (1).

*P. aeruginosa* can cause localized or systemic diseases in young and growing poultry and invade fertile eggs causing death of embryos and newly hatched chicks; this suggests a possible egg borne infection (John Barnes, 1997). *P. aeruginosa* were detected from yolk sac samples by 25 (12.5%) and confirmed positive sample by detection of *groE* gene fig (3).

*S. aureus* infection has become an increasingly grave problem in industrialized poultry farming. Staphylococcal infections including, synovitis with arthritis, osteomyelitis, dermatitis, endocarditis, septicemia, wound infection and omphalitis (Bergmann et al. 1980) *S. aureus* was detected in 10 samples with recovery percent (5%) , which indicated that *S. aureus* can be considered as one of important etiological agent of yolk sac infection (Deeming., 1998).

The isolation percent of *Salmonella* spp was too far extent is low (2.5%) in comparison to previous studies as Nasrin et al. 2012 showed the highest prevalence for *Salmonella* on both chicks aged 1-3 days and 4-7 days (68 and 54.3%, respectively).

The use of antibiotic feed additives in poultry results in a high prevalence of resistance among their enteric bacteria, with a consequent emergence of antibiotic resistance in zoonotic enteropathogens (Osman et al. 2012, Osman & Elhariri 2013). The antimicrobial sensitivity patterns were detected for all bacterial genera; the highest resistance patterns were exhibited by *P. aeruginosa* followed by *Salmonella* spp. then *E. coli* and finally *S. aureus* (Table 2).

*P. aeruginosa* isolates exhibited high resistance patterns for amoxicillin, ampicillin, nalidixic acid, gentamicin, streptomycin, tetracycline, and erythromycin with percent 100 % . Always, the high level of resistance pattern for *P. aeruginosa* isolates from different animal species in Egypt is reported (Osman et al. 2012, Elhariri et al. 2017) or from baby chicks (Walker et al. 2002). In the same manner, *Salmonella* isolates produced a high resistance pattern to most examined antimicrobial drugs except streptomycin, tetracycline and norfloxacin.

All *S. aureus* isolates were completely resistance to sulphamethazone, ampicillin and nalidixic acid (100 %) as shown in table (2) .These results may be agree or disagree with many

authors due to the difference in many conditions surrounding hatcheries where this study was made and also because of the organism's propensity to acquire antimicrobial resistance, so it is important to continually monitor antibiotic susceptibilities of clinical isolates.

## CONCLUSION

The emergence of multiple resistance bacterial pathogens associated with omphalitis is a highly risk factor to the poultry industry. The antimicrobial therapy of no significant value as the recovered chicks will be uneven and will not become a viable commercial proposition. Control is best accomplished by giving the most possible brooding conditions and ensuring that only healthy chicks from well-managed breeding flocks and hatcheries are acquired. The most imperative angles are good hatching egg hygiene on the breeder farm and effective disinfection programs in the hatchery

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

## AUTHOR CONTRIBUTIONS

NE & SAN designed the experiments and ME wrote and reviewed the manuscript. ZAS, and RE performed all The experiments. All authors read and approved the final version.

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